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"Celebrating 50 years of the European Aquaculture Society"

Welcome to Aquaculture Europe 2026



Dear Colleagues and Friends,

It is my great pleasure to welcome you to Aquaculture Europe 2026, taking place in the beautiful city of Ljubljana. This year's theme, "*Aquaculture in Global Change*," could not be more timely. Sitting at the heart of central and southern Europe, bordered by Italy, Croatia, Hungary and Austria, Slovenia offers a perfect point from which to explore adaptation of both freshwater and marine aquaculture to climate effects, understand species resilience, and highlight the health, welfare, nutrition and breeding advances that will carry our sector forward. Over this week in Ljubljana, our plenary speakers, scientific sessions, industry and innovation forums, and exhibition will bring together the full breadth of European and international aquaculture to share ideas, forge collaborations, and chart a course for a more resilient, sustainable future. Again (and we can only be happy for that!) Aquaculture Europe promises a lot, with more than 730 abstracts received, and 170 booths at the exhibition

This edition of Aquaculture Europe also carries special significance: 2026 marks the 50th anniversary of the European Aquaculture Society. Since 1976, EAS has brought our community together in pursuit of a sustainable European aquaculture sector, and Ljubljana gives us the perfect setting to celebrate this milestone together.

I warmly invite all EAS members to join our special EAS50 General Assembly on Tuesday, 29 September, 17:30–18:30 (Gallery 1, Kocka Hall), where we will look back on five decades of achievements and formally welcome the incoming EAS Board. The Assembly will be followed immediately by a dedicated EAS50 reception and buffet dinner for all members — a chance to celebrate this anniversary, and the community that made it possible, together. As key actors of this, we will be happy to share this moment with eleven of our Past Presidents and eight of our Honorary Life Members.

On behalf of the EAS Board, thank you for being part of this milestone year. I wish you an inspiring, productive and enjoyable conference in Ljubljana.

Warm regards,

Marc Vandeputte

European Aquaculture Society President 2024-2026

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ABSTRACTS

FUNGAL SINGLE-CELL PROTEIN PRODUCTION FROM MACROALGAL WASTE: ON THE ROAD TO SUSTAINABLE AQUAFEEDS

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Background

Feed costs represent a significant portion of the total production expenses in the aquaculture industry. These costs may range from 75% to 90%, especially due to the latest price increase on raw materials, such as fish meal and oil, that are now perceived as unsustainable due to their limited availability (Ariffin Hidir *et al.*, 2026). The use of plant-based alternatives, such as soybean meal, as aquafeed ingredients has reduced reliance on marine-derived ingredients but has also raised ecological and nutritional concerns. This scenario exposes the lack of sustainable, nutritionally adequate, and scalable substitutes for traditional aquafeed proteins, underscoring a broader challenge for the sector (Aziz Atnafu *et al.*, 2026). One promising solution is to use single-cell proteins (SCPs) obtained from the fermentation of filamentous fungi. Their consistent nutritional value (40–60% protein), content of essential amino acids, polyunsaturated fatty acids, vitamins, and minerals, together with the presence of functional ingredients (e.g., mannanoligosaccharides and β -glucan), may provide further health benefits in fish, such as enhancing growth performance and antioxidant defences and, consequently, disease resilience. Moreover, the ability of the fungi to use diverse low-cost substrates and reduce land use and water requirements makes this protein source a potentially sustainable alternative. This includes the possibility of macroalgae waste fermentation, which is widely available in European sea basins and, when discarded from human-destined industries, can be transformed into a value-added product. The AlFunFeed project adopts a holistic perspective linking marine resource valorization with aquaculture nutrition, addressing one of the major challenges for the aquaculture industry: the development of a reliable and sustainable alternative source of protein for aquafeeds.

Objective

AlFunFeed aims to develop and validate SCPs produced from macroalgal processing residues as a sustainable and alternative aquafeed ingredient. The project will optimise SCP-based feed formulations, evaluate their effects on growth performance, nutrition, and fish health in European seabass, while assessing their economic feasibility and environmental sustainability.

Methodology

Fungal SCP variants produced from the fermentation of macroalgal residues will be incorporated into experimental diets for European seabass (*Dicentrarchus labrax*). CIIMAR will perform a two-stage *in vivo* validation, including a pilot feeding trial to screen different SCP sources followed by a validation trial to determine the optimal dietary inclusion level (i.e., 15%, 20%, and 25% SCP). Fish performance will be evaluated through growth, feed utilization and nutrient digestibility, while health effects will be assessed using haematological, humoral, and mucosal immune biomarkers, gene expression analyses and bacterial challenges with a relevant aquaculture pathogen, *Tenacibaculum maritimum*. The integration of these nutritional, physiological, and immunological parameters will enable the identification of SCP-incorporated formulations that can modulate and enhance fish performance and health, thereby contributing to more sustainable aquaculture.

Regarding other partners' roles in AlFunFeed, ODS will be responsible for SCP production and characterisation, the University of Vigo will coordinate and assess digestive function, nutritional profiling and appetite, the University of Thessaly will study the microbiota alterations, and the Università Politecnica delle Marche will be responsible for fish welfare assessment.

(Continued on next page)

Expected outcomes

The project aims to identify and validate fungal SCP derived from macroalgal residues that, when incorporated into aquafeed, can improve European sea bass growth, welfare, and disease resilience. This will contribute to welfare-focused and environmentally conscious aquaculture, to waste reduction, to decreasing the pressure on marine resources, and to lowering the carbon and nutrient footprints of the aquafeed and aquaculture industry.

Acknowledgments

This project has received funding from the European Union's Horizon Europe research and innovation programme (SBEP2024-794 AIFunFeed), and national funds through FCT - Fundação para a Ciência e a Tecnologia, I.P., within the scope of SBEP/0002/2024. <https://alfunfeed.eu/>

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A MEASUREMENT METHOD WITH COMPUTER VISION FOR INDIVIDUAL FEED INTAKE IN GROUP CONDITION IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

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Introduction

Individual feed intake (IFI) measurements in aquaculture is challenging to measure because of the nature of the culture system where the fish are reared in group condition. Measuring IFI can enable several advancements in aquaculture development to reduce feed cost and environmental impact, but mainly it is required in selective breeding to select feed conversion ratio (FCR) as a trait. Indeed, the ability to measure IFI is the current limiting factor in achieving appropriate estimation of breeding values for FCR. Current methods are invasive with X-ray imaging and RFID-tag in feeding devices, or neglect social behaviour with individual tanks. Here we propose a new approach in measuring IFI at group condition using computer vision (CV). CV enables the automated interpretation of complex visual data to derive actionable biological insights. The idea is to have a practical method for measuring IFI in groups, to maximize the fish capabilities in feed conversion with selective breeding.

Method

European seabass (200g average) were stocked in 240L aquaria at densities of 10, 15, and 20 individuals to facilitate CV development. Over 5 days, fish were filmed daily for one hour during *ad libitum* feeding, using a custom distributor that placed pellets near the front glass to optimize video recording of feeding events. Initial and final biometry were recorded, and individual fish were filmed separately beforehand to create a gallery set for re-identification (Re-ID) comparison. To detect feeding events, two YOLOv8 models were developed using 300 randomly extracted frames each, for “PELLET” and “OPEN MOUTH” occurrences. The system identifies a feeding event when a tracked pellet disappears and a proximity search simultaneously detects an “OPEN MOUTH” bounding box. This dual-condition logic ensures accurate consumption tracking within the video data. Individual feeding contributions were quantified using two distinct Re-ID approaches derived from the FISHNESS project dataset (Crestel et al., 2026). The first utilized Vision Transformers (ViTs) trained on over 18090 (divided to 80/20 train and test set) full-body images of European seabass representing 4944 individual fish photographed at 2 to 5 different ages. The second employed a Convolutional Neural Network (CNN) focused specifically on the head region, defined from the snout to the opercula. These models allow the CV system to attribute specific feeding events to individual fish within the experimental groups. In parallel several supplementary models are also built in order to integrate the feeding event with the Re-ID model. We built the “EYE”, “FISH” and “HEAD” detection models are built with only 100 frames each with YOLOv8 architecture. The “EYE” detection are used in order to track the fish and assign the Re-ID model identification. The “FISH” model are used to generate crop of the whole fish body and send it to the Re-ID model for identification. The “HEAD” model is similar but crops the head part only. Finally, the whole pipeline works by tracking the eyes of the fish in the aquarium. Where there are head or the whole fish detection by the “HEAD” and “FISH” models within the tracked eye, the bounding boxes are used to crop the frame and send it to the Re-ID model for identification. The IDs are then assigned back to the tracked eye. Then, when there are feeding events detected by the logic explained before, the feeding events are attributed to the closest eye that is detected, tracked and identified. Validation is done manually by carefully evaluating the feed intake output video. Evaluation metrics are based on F1 score as a standard metric. F1 score are calculated

$$\text{as } F1 = \frac{2 \times \text{Precision} \times \text{Recall}}{\text{Precision} + \text{Recall}} \text{ with } \text{Precision} = \frac{TP}{TP + FP} \text{ and } \text{Recall} = \frac{TP}{TP + FN}. \text{ TP means true positive (feed}$$

intake happened and detected), FP means false positive (there are no feed intake but it was detected) and FN means false negative (there are feed intake but it is not detected). For the Re-ID, rank-1 (the percentage of correct prediction of the 1st rank) are used for evaluation.

(Continued on next page)

Results and Discussion

All of the detection models were trained successfully with F1 score of >0.8 . The head Re-ID model with CNN training resulted in rank-1 score of 100% highlighting a probability of overfitting, that requires refinement with more training dataset or parameters tuning. Meanwhile, the whole fish Re-ID model training with ViTs resulted in rank-1 score of 95.7%. This score is also high but not as perfect as the head CNNs Re-ID model. Both model are tested on images from the 80/20 data split. We continue to build the whole pipeline taking into account the fact that the modular nature of the pipeline allows for the seamless integration of refined Re-ID architectures as larger, more diverse datasets become available. The whole pipeline was then applied with the logic explained in the Method section. The preliminary results shows that in Aquarium 1 (with 10 fish), 470 minutes of videos were analyzed, 619 feed intake events detected with 85% attributed to an ID. Fish with ID 711 had the highest feed intake with 186 (30% of the total) pellets eaten while fish with ID 622 had the lowest feed intake with only 2 (0.3%) pellets eaten. Other videos are being processed and analyzed. Preliminary validation of the feed intake resulted in F1 score of 0.8 proving that the pipeline is working. The current analyses shows promising results for this pipeline to be used for IFI measurement in group condition of European seabass. Several limitations such as lack of objective validation method for some modules, model overfitting problems and video processing time are areas that need further development. However, this result acts as a proof of concept that measuring IFI with computer vision is possible and with future developments, it can be a reliable method. Several indirect measurements which have been hypothesized to correlate with IFI (e.g., weight loss at fasting, growth rate, lipid content) can also be proven by this method. Overall, this is the first step in creating non-invasive, high-throughput, group conditioned IFI measurement with CV.

Acknowledgment

This work is part of the SEPIAA project (funded by France 2030 and BPI), with experimental support from the Ifremer PEMP station in Palavas-les-Flots, France.

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REDUCED CRUDE PROTEIN DIETS IN FERTILIZED EARTHEN PONDS: GROWTH PERFORMANCE, FEED UTILIZATION AND ECONOMIC RETURNS OF NILE TILAPIA *Oreochromis niloticus* IN SUBSAHARAN AFRICA

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Background

Feed protein is the single largest cost driver in pond tilapia aquaculture across sub-Saharan Africa, accounting for 50–70% of variable production costs. In Ghana, commercial diets formulated at 30% crude protein (CP), the prevailing market standard, produce farm-gate tilapia prices roughly five times higher than in Egypt, where 25% CP grow-out feeds are routine practice (El-Sayed, 2013). A 25% CP grow-out pellet remains absent from the Ghanaian commercial market. Fishmeal prices have risen by approximately 30–40% over the past decade internationally, further widening the cost burden for smallholder pond farmers. In fertilized pond systems, natural phytoplankton can supply 50–70% of total protein requirements even when complete commercial diets are provided (Schroeder, 1980), yet controlled field evidence validating reduced-CP diets under commercial pond conditions in West Africa has been lacking.

Materials and Method

A 16-week pond trial was conducted at Konama Farms in Kokoben, Atwima Nwabiagya District, Ashanti Region, Ghana, comparing three commercial extruded floating pellet diets (25%, 28% and 30% CP; 4.5 mm pellet). Sexreversed *Oreochromis niloticus* fingerlings (~60 g) were stocked at 1.6 fish m⁻² across eight fertilized earthen ponds (330–630 m²). Ponds were pre-fertilized with mono-ammonium phosphate (MAP; 2 g m⁻²) and urea (3 g m⁻²) prior to stocking and supplementarily when Secchi depth exceeded 40 cm. Fish were fed twice daily at 2–3% body weight, adjusted after monthly sampling. Growth performance, feed utilization, water quality and enterprise economics were assessed monthly. Data was analyzed by one-way ANOVA with Tukey HSD post-hoc test ($P < 0.05$) using GraphPad Prism v5 and Minitab v17.

Results and Discussion

Fish fed the 25% and 30% CP diets achieved statistically equivalent and significantly superior growth compared with the 28% CP group ($P < 0.05$; Table 1). Specific growth rates (SGR) were 1.27, 1.21 and 1.29 %/day for 25%, 28% and 30% CP, respectively. Feed conversion ratios (FCR) did not differ significantly across treatments (1.99–2.09). Phytoplankton-derived natural food, evidenced by significantly higher chlorophyll-a concentrations in 25% CP ponds (495 ± 42 vs. $427\text{--}441 \mu\text{g L}^{-1}$; $P = 0.0149$), appeared to compensate for the lower dietary protein level, consistent with Schroeder (1980) and Frimpong et al. (2014). Ammonia concentrations remained below the 0.20 mg L^{-1} stress threshold in all treatments (Bhatnagar & Singh, 2010). All other water quality parameters were within acceptable ranges for tilapia culture.

The comparatively lower growth in the 28% CP group may be attributed to two factors. Elevated recruitment biomass from incomplete sex reversal intensified intraspecific competition between grow-out stock and unintended recruits, suppressing individual growth rates. Additionally, the 28% CP diet may have been less complementary to the amino acid profile of available natural food than the 25% or 30% CP diets, reducing effective protein utilization. The interaction between dietary and natural food protein sources in fertilized ponds needs further investigation across a broader CP range.

The 25% CP treatment generated approximately 19% return on investment, compared with 6–9% for the higherprotein alternatives. Fishmeal prices currently stand at approximately \$1,700–2,000 per tonne internationally, substantially above levels recorded in the early 2010s, which amplifies the per-kilogram savings achievable by reducing dietary CP. At current input prices, switching from 30% to 25% CP feed saves approximately $\$0.35 \text{ kg}^{-1}$ of feed, translating to over $\$0.33 \text{ kg}^{-1}$ of fish produced across a full grow-out cycle. Applied to Ghana's estimated 80,000 ha of viable pond area, this represents a sector-wide annual cost reduction in the order of tens of millions of dollars.

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Table 1. Growth performance, feed utilization and economic returns of Nile tilapia (*Oreochromis niloticus*) fed diets with varying crude protein (CP) levels in fertilized earthen ponds.

Parameter	25% CP	28% CP	30% CP
Growth performance			
Final body weight (g)	299.6 ± 20.3 a	277.1 ± 11.6 b	305.4 ± 12.6 a
Weight gain (g)	239.8 ± 10.8 a	217.3 ± 2.04 b	245.6 ± 3.04 a
SGR (%/day)	1.27 ± 0.03 a	1.21 ± 0.01 b	1.29 ± 0.01 a
Daily weight gain (g/day)	1.89 ± 0.09 ab	1.74 ± 0.03 a	1.94 ± 0.02 b
Survival rate (%)	93.7 ± 3.79	93.7 ± 5.03	93.0 ± 0.00
Feed utilization			
Feed conversion ratio	2.07 ± 0.10	2.09 ± 0.37	1.99 ± 0.13
Protein efficiency ratio	1.93 ± 0.10	1.74 ± 0.31	1.73 ± 0.11
Feed intake (g/fish/day)	2.42 ± 0.55	2.61 ± 0.28	2.62 ± 1.34
Economic analysis(per hectare)			
Feed cost (USD kg ⁻¹ feed)	\$0.51	\$0.72	\$0.86
Feed cost as % of total cost	40%	48%	51%
Production cost (USD kg ⁻¹ fish)	\$2.42	\$3.10	\$2.76
Net profit (USD ha ⁻¹)	\$1680 (~19%)	\$617 (~6%)	\$1034 (~9%)
a, b: values in the same row with different superscripts differ significantly ($P < 0.05$, Tukey HSD). Feed utilization parameters: no significant differences among treatments ($P > 0.05$). Initial body weight: 59.78 ± 9.52 g (all treatments). Stocking density: 1.6 fish m ⁻² ; fertilized earthen ponds, Kokoben, Ghana. USD conversion at GHC 3.95 per USD.			

Conclusion

Nile tilapia in fertilized earthen ponds achieve commercially equivalent growth and feed utilization on 25% CP commercial diets as on the 30% CP industry standard, at substantially lower cost and with no detrimental effect on water quality. These results support the commercial introduction of 25% CP grow-out pellets in Ghana and comparable sub-Saharan African production systems, with direct relevance to the current global push for sustainable, cost-competitive aquaculture feed formulation.

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EVALUATING THE EFFICIENCY OF SELECTIVE BREEDING PROGRAM IN COMMON CARP UNDER FISHPOND MANAGEMENT: A COMPARATIVE STUDY IN PROGENY ORIGINATING FROM MALES OF SELECTED AND NON-SELECTED POPULATIONS OF AMUR MIRROR CARP

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Introduction

Selective breeding is applied in aquaculture to enhance desirable performance traits. In Czechia, an experimental selective breeding program for faster growth in common carp, Amur mirror carp (AMC), reared under fishpond management started in 2017. In 2022, we decided to compare performance and morphometry of progeny established from males of the first selected generation (G1) and non-selected one (G0) and to match realized heritability and genetic gain with those estimated in previous study (Prchal et al., 2024).

Materials and Methods

A total of 50 males (25 from selected and 25 from non-selected population) and 15 females of non-selected population were used to establish experimental stocks via artificial spawning and individual sperm insemination. Fertilization rate, hatching rates (from inseminated and fertilized eggs), and percentage of malformed larvae were compared between the two groups. Phenotyping was performed four times during the 2-year fishpond culture period at the end of two growing seasons and two overwintering periods. Data on growth-related traits and survival were determined and corrected using an internal control (Linhart et al., 2002). In two-year-old carp, muscle fat content, fillet yield predictors E8 (abdominal fillet thickness) and E23 (abdominal depth) were also measured. The genetic gain and realized heritability (h^2) of the weight at the end of the second overwintering (C2A–C2S) were calculated. Fish digitization was done at the end of the second growing season. Seventeen landmarks were marked using ImageJ and a specifically designed macro to align each fish horizontally and collect the landmark coordinates. Truss network of relative values, 36 in total, was generated. All data were statistically analyzed using R Studio and Statistica.

Table 1: Growth performance evaluation

Period	Growth-related traits	Treatment groups	
		Selected	Non-selected
C0–C1A	Weight (g)±S.D.	15.91±2.72 ^a	14.99±4.11 ^b
	SL (mm)±S.D.	76.87±4.55 ^a	74.70±6.55 ^b
	K±S.D.	3.22±0.24 ^a	3.37±0.52 ^b
C1A–C1S	Weight (g)±S.D.	16.66±3.79 ^a	13.74±3.73 ^b
	SL (mm)±S.D.	76.01±5.27 ^a	71.24±6.31 ^b
	K±S.D.	3.43±0.29 ^a	3.49±0.37 ^a
C1S–C2A	Weight (g)±S.D.	352±117.0 ^a	318±88.7 ^b
	RBW±S.D.	0.19±0.01 ^a	0.19±0.01 ^a
	K±S.D.	2.64±0.22 ^a	2.71±0.23 ^b
	E8/E23±S.D.	0.128±0.01 ^a	0.134±0.01 ^b
	Muscle fat (%)±S.D.	2.58±0.89 ^a	2.69±0.97 ^a
C2A–C2S	Weight (g)±S.D.	332±96.2 ^a	306±83.4 ^b
	K±SD±S.D.	2.65±0.23 ^a	2.71±0.35 ^b
	Muscle fat (%)±S.D.	2.73±0.72 ^a	2.89±0.74 ^b

K (Fulton's condition factor) = $10^5 \cdot \text{BW (g)} / \text{SL}^3 \text{ (mm)}$; **C0–C1A**: the first growing season; **C1A–C1S**: the first overwintering; **C1S–C2A**: the second growing season; **C2A–C2S**: the second overwintering; **SL**: Standard length; **K**: Fulton's condition factor; **S.D.**: Standard deviation; **E8/E23**: Fillet yield predictor. Groups with non-identical alphabets are significantly different at $p < 0.05$.

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Results and Discussion

The analyses on reproductive success showed the hatching of inseminated eggs ($p < 0.05$) and fertilized eggs differed significantly ($p < 0.05$) between the groups in favor of the selected line. However, the observed fertilization rate (selected = 84.13% and non-selected = 86.51%) and larval abnormalities (Selected = 2.81% and non-selected = 2.72%) were insignificant.

Cumulative survival rate from the larvae to age of two years (C0 - C2S) was significantly higher in the selected line ($20.18 \pm 1.49\%$) compared with the non-selected line ($12.44 \pm 1.49\%$) ($p < 0.05$), representing an improvement of approximately 62% relative to the non-selected line. This result indicates that selective breeding for faster growth rate may have indirect effect on fish survival. The growth evaluation results (Table 1) demonstrate that the selection program exerted a clear and consistent positive effect on weight and SL, throughout the experimental period. The selected group consistently outperformed the non-selected group in weight ($15.91 - 332\text{g}$ vs $14.99 - 306\text{g}$) with 17% genetic gain of the final weight and a moderately high realized h^2 of 0.355. The genetic gain was in accordance with previous study (Prchal et al., 2024). Interestingly, the selected group also exhibited significantly lower fat content ($2.58 - 2.73\%$) compared to the unselected group ($2.69 - 2.89\%$). This finding reflects a favorable shift in nutrient partitioning, whereby selected individuals allocate proportionally more energy toward lean tissue (muscle and skeletal growth) rather than fat storage as improved growth often increases fat content (Bauer & Schlott, 2009).

Morphometric analysis using truss network of corrected relative values comparison revealed that 10 out of the 36 landmarks examined showed statistically significant differences ($p < 0.05$) between the groups. However, the differences, even if significant, were marginal. Nevertheless, it will be interesting to monitor the changes in morphometry of carp stocks over the generations of selective breeding. This study confirms the efficacy of a selective breeding program for a higher growth rate.

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GROWTH PERFORMANCE, BIOCHEMICAL, ANTIOXIDANT STATUS AND HISTOPATHOLOGY CHANGES OF *Clarias gariepinus*, RAISED IN A BIOFLOC MEDIUM UTILIZING NATURAL CARBON SOURCES

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Introduction

Aquaculture has persistently developed globally under best practices. A good health status from the function point of view is preponderant for both cultured and wild fish species in early development (Mota *et al.*, 2019). Poor health precludes the reduced exponent of the fish to perform its physiological functions, conform to stressful conditions, and prevent disease (Caipang *et al.*, 2015). Lofty intake of protein above 25% by both fin and shellfish is remarkably excreted as ammonia-nitrogen and influence the quality of water. When inorganic nitrogen, total suspended solids (TSS) are high in concentration in culture water, it leads to death of culture species, diseases, accumulation of harmful residues in culture systems (Karunaarachchi *et al.*, 2018), and obstruction of the growth of global aquaculture market (Caipang *et al.*, 2015). To promote efficient fish production, recycling aquaculture system though expensive to maintain, is most preferable. Therefore, it is apropos to utilize non-expendable ecological clues to resolve the problems associated with cost, poor fish production, etc. However, inappropriate husbandry conditions such as poor water quality, crowding leading to accentuated social interactions between similar schemes present in the culture strategies are a potential threat to animal health/welfare. Therefore, biofloc technology (BFT) provides an alternate solution as a consequence of practicing zero-water exchange, waste nutrient recycling (Bossier and Ekasari, 2017) regulating water qualities by microbial communities, promoting fish growth (Hamidaghli *et al.*, 2019) with reduced environmental impact (Bossier and Ekasari, 2017) for sustainable aquaculture. It is a more beneficial system when heterotrophic bacteria, a nitrogen conversion agent is stimulated by adjusting the C:N ratio in the culture system by the addition of carbonaceous plant-based material or equivalent inorganic carbons for sustainable fish production to achieve sustainable development goals (Bossier and Ekasari, 2017). It has also offered practical solutions to effective water quality control (Avnimelech, 2007) and improvement of fish growth in the healthy culture system (Lananan *et al.*, 2014).

Materials and Methods

A 28 days preliminary trial was conducted to evaluate the affect of biofloc technology application on *Clarias gariepinus* juveniles with emphasis on the growth performance and impact on physiological status. A total of two hundred and seventy juvenile *Clarias gariepinus* were randomly distributed into six plastic bioreactors groups (A-F) in triplicates with equal volume of inoculum, maintained in 30 L tank with constant aeration. Addition of organic carbon sources (CN 10:1, 20:1) were evaluated to stimulate rapid growth of microbial dynamics biomass whereas, no carbon sources were added in the control systems. The CN ratios (cassava and wheat) of 10:1 and 20:1 were; cassava (CA 10:1, 20:1) groups C, D, and wheat flour (WH10:1 and 20:1) groups E and F, respectively. Meanwhile, the normal controls, group A (inoculum, no carbon addition) and B (no inoculum, no carbon) had 50% water exchange and received only fish feed at 3% body weight.

Result

The physicochemical parameters maintained the tolerable limit for catfish farming. Significant weight gain, specific growth rate, Length Weigh Relationship (LWRs), survival rate, and floc volumes were observed at the end of experiment. Observed changes in the catalase, superoxide dismutase, glutathione peroxidase, and malondialdehyde activities in the gill were between day 14 and 21. Significant levels (p1 of *C. gariepinus* juveniles within 28-days. inclusion of C:N ratio of cassava and wheat flour in biofloc tank is beneficial for the growth and health parameters of *Clarias gariepinus*.

Discussion

The adjustable C:N ratio of cassava and wheat flour in flocculation is essential, efficient, and indicated a positive reduction of inorganic nitrogen and noxious metabolites produced in the culture system of African catfish, *Clarias gariepinus*. It is pertinent to observe the peculiar attributes of natural carbohydrates sources such as locally available, not harmful to the cultured fin and shellfish, and cheap. Fish culture using BFT is more beneficial for better growth performances with a reduced pathophysiology change. CA 10: 1 and WH 20:1 is better utilized in *Clarias gariepinus* culture compared to CA 20:1 and WH 10: 1. There is a need to investigate other cheaper sources of carbohydrates such as white yam tuber (*Dioscorea rotundata*), water yam (*Dioscorea alata*), cocoyam (*Xanthosoma sagittifolia*, *Coloca sia esculenta*) and relevant agricultural by-product as natural carbon sources in culture of finfish in a biofloc media.

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PREPARING A FEED FROM AGRI-FOOD INDUSTRIES BY-PRODUCTS FOR *Hediste diversicolor*

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Introduction

Hediste diversicolor is characterized by its ability to elongate and desaturate long-chain polyunsaturated fatty acids from substrates of low nutritional quality (Villena et al., 2025). This, alongside its high protein content, makes it a potential alternative ingredient for the aquafeed industry in a context of sustainability and circular economy. The production of *H. diversicolor* biomass for use as feed in aquaculture—whether in its raw form or as meal—requires optimizing its cultivation, which makes it essential to have access to a suitable feed at the lowest possible cost. This study evaluates diets formulated from agri-food industry by-products in terms of growth performance and the resulting fatty acid profile.

Materials and methods

After a preliminary selection process of raw materials derived from waste products from the wine, beer, cider, and aquaculture industries (Aguado-Giménez et al., 2025), along with the inclusion of other raw materials typical of the aquaculture feed industry (to ensure an adequate protein intake), we formulated four diets for *H. diversicolor*. Table 1 shows the proportions of each raw material used in each of the four diets and their basic nutritional composition.

A growth trial was performed using ad hoc experimental units (EU) and following the experimental apparatus described in Aguado-Giménez et al. (2023), at a density of 2,000 individuals m⁻², a temperature of 18 °C, and a salinity of 35‰, for 2 months. Specific growth rate (SGR), Survival Rate (SR) and the resulting fatty acid (FA) profile were assessed.

Results and Discussion

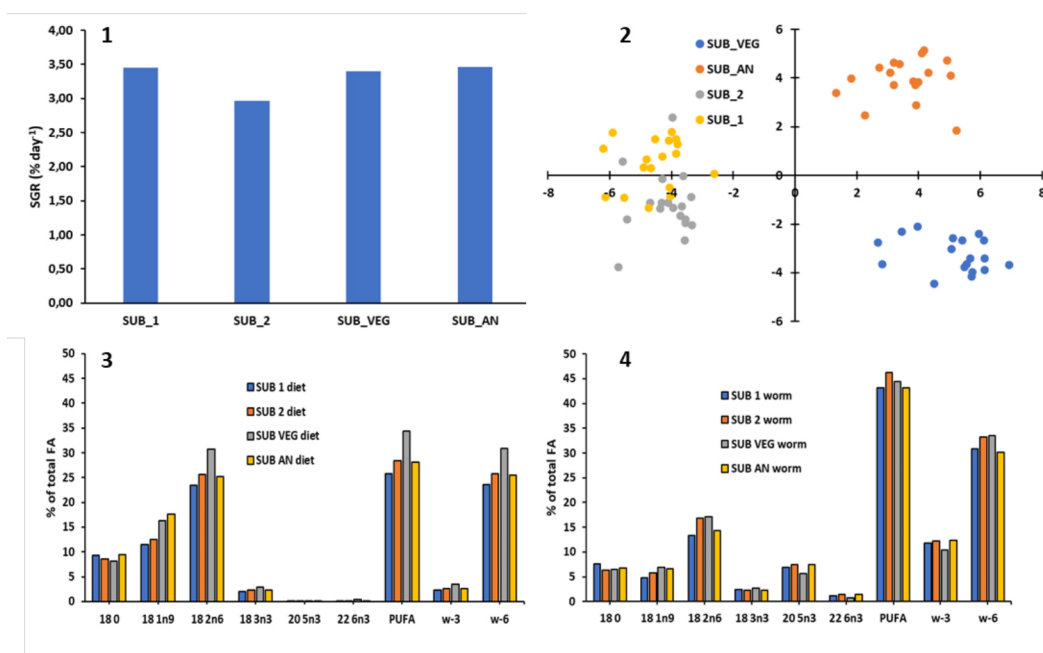
SR was very close to 100% with all diets. SGR was significantly lower with the SUB_2 diet. The other diets resulted in very similar SGR values, which were slightly higher with SUB_1 and SUB_AN (Figure 1). *H. diversicolor* significantly transformed the dietary fatty acid profile into biomass richer in PUFAs and omega-3 fatty acids (Figures 2-4). Polychaetes fed the SUB_1 and SUB_2 diets had a fatty acid profile distinct from that obtained with the other diets (Figure 2), characterized by a higher content of LC-PUFAs, particularly those of the ω -3 series. In general, worms fed any of these diets showed a significant increase in the proportion of PUFAs, particularly EPA and ω -3 series as a whole, compared to their respective diets (Figures 3-4).

Of the four formulated diets, diet SUB_1 was selected to enhance *H. diversicolor* biomass production for subsequent experiments involving its inclusion in marine fish feed. This diet provided the best balance between growth performance, fatty acid profile, and compliance with circular economy criteria.

Table 1: Ingredient and nutritional composition (%) of the four formulated feeds. RoWL: rose wine lees; BSG: brewer’s spent grain; BFL: biofloc waste.

	RoWL	BSG	BFL	Soy meal	Meat meal	Protein	Lipids
SUB_1	70	20	10	-	-	31.89	4.33
SUB_2	70	30	-	-	-	33.22	4.83
SUB_VEG	50	20	10	20	-	34.21	5.52
SUB_AN	50	20	10	-	30	34.11	5.70

(Continued on next page)



Figures 1-4: 1: SGR obtained with the four formulated diets; 2: and discriminant analysis for the fatty acid profile of the worms fed with the four diets; 3: Fatty acid profile of the four diets; 4: Resulting fatty acid profile of worms fed the four diets.

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EGG DISINFECTION IN *Chelon labrosus* USING A IODINE-BASED PRODUCT.

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Introduction

The thick-lipped grey mullet (*Chelon labrosus*) is a low trophic candidate for the diversification of aquaculture species in Europe. As in other marine species, considerable mortalities have been reported during larval rearing. Some authors have linked survival problems to the presence of microorganisms (Kusuda et al., 1986). Egg disinfection is a routine procedure used to reduce the bacterial, viral, and fungal load on the surface of eggs in species such as salmon, trout, turbot, etc. Disinfection protocols must be optimized for each species. Numerous studies support the effectiveness of iodine as a disinfectant (Hirazawa et al., 1999).

Material and methods

Eggs were obtained through natural spawning induced by hormonal treatments applied to females (Besbes et al., 2020). Eggs were collected using 335 μ m mesh nets at the outlet of the broodstock tanks. From a single spawn, batches of 5 ml of eggs, were used for disinfection trials. Disinfection process was carried out using an iodine-based solution (1.116 % p/p) in 1 L beakers. The procedure was: 100 ml per liter concentration for 10 minutes contact time, and three subsequent rinses of 5 minutes each with clean water. The eggs were at an advanced morula stage prior to the onset of gastrulation at the time of disinfection (6–10 hours post-fertilization at 16°C). The experimental control consisted of eggs from the same spawn subjected to the same procedure but without the use of iodine.

Two assays were performed using both disinfected and control eggs:

1. **Hatching rate and larval survival in 3 ml-well cell culture plates of control and disinfected eggs:** The plates contained embryos incubated with 2ml of sterile sea water that were checked to assess hatching and larval survival rates. The hatching rate was calculated as the total number of hatched larvae divided by the total number of incubated eggs, and larval survival was evaluated daily.
2. **Comparison of the microbiota associated with control and disinfected eggs:** Blood agar Petri dishes supplemented with NaCl were prepared from the egg batch: three dishes containing 60 untreated eggs per plate and three dishes with 60 disinfected eggs per plate. Samples were analyzed for specific identification using the Vitek 2 system (Biomérieux, Madrid, Spain), which employs colorimetric reagent cards that are incubated and automatically interpreted after performing a Gram stain, a catalase test using 3% hydrogen peroxide, and a coagulase test with rabbit plasma.

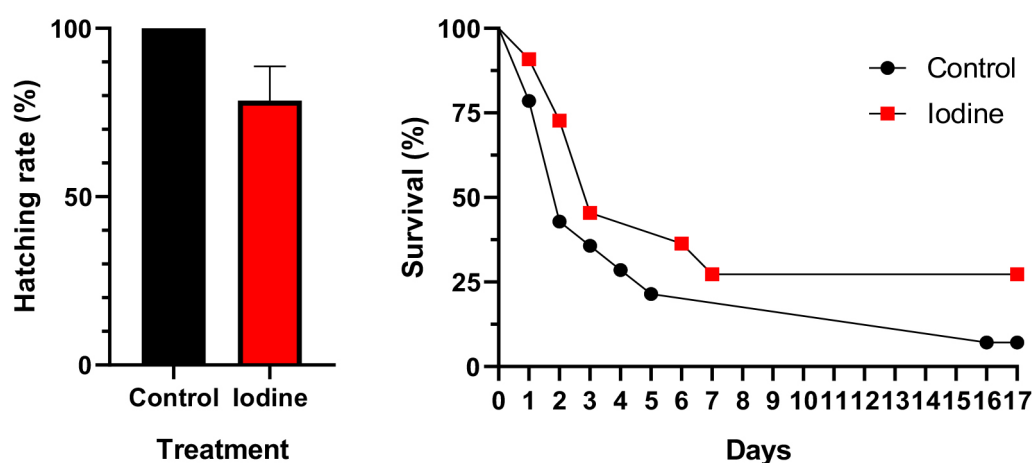


Figure 1. A. Hatching rates of untreated (control) eggs and eggs disinfected with iodine. The figure shows the results as the mean $\pm$ standard error. B. Kaplan–Meier survival curves of embryos disinfected and not disinfected (Control) with iodine.

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Results and discussion

As shown in figure 1, no significant differences were found in either the hatching rates (Mann-Whitney, $p>0.05$) or the survival curves (Mantel-Cox, $p>0.05$) between control eggs (untreated) and those disinfected with the iodine solution. Regarding the identification of flora associated with the eggs and the effect of disinfection, all control batches tested were positive—by identification using the Vitek method—for *Aeromonas salmonicida* / *Bordetella* spp. In contrast, all batches disinfected with Buffodine, following the manufacturer's instructions, showed a complete absence of bacterial growth.

In conclusion, the authors recommend a disinfection with 100 ml l⁻¹ of iodine-based solution for 10 minutes followed by three rinses with seawater to reduce significantly the bacterial load in *Chelon labrosus* eggs without affecting the hatching rate or subsequent survival of the larvae. This methodology is simple and easy to implement as a routine. However, these are the first published data about disinfection in this species, and it would be desirable to test the efficacy of this compound for viruses and fungi, the efficacy of other compounds, or the possible adverse effects of disinfection at different embryonic stages.

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MOLECULAR DETECTION OF SULFONAMIDE (Sul1) RESISTANT GENE ISOLATED FROM BACTERIA IN *Chrysichtys nigrodigitatus* (Lacepede, 1803) FROM OGUN RIVER, AKOMOJE IN ABEOKUTA, OGUN STATE

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The study was carried out to investigate the antibiotic sensitivities of bacteria isolated and identified from *Chrysichtys nigrodigitatus* and their antibiotic susceptibility pattern determined. A total of 45 samples of skin, gill and intestine of *Chrysichtys nigrodigitatus* were obtained from Ogun River, Akomoje in Abeokuta, Ogun State and analyzed microbiologically. The mean values of characteristics measured for the body weight, total length, and standard length for the 15 fish samples are 147.99 ± 66.41 , 26.55 ± 5.55 and 20.05 ± 4.13 respectively while the physical and chemical parameters of Ogun River, Akomoje during the study is presented as follows; Water Temperature (29.0°C), pH (7.8), Dissolved Oxygen (4.9mg/l) and Ammonia (2.3ug/dL). The total mean bacteria count in the gill samples was $1.06 \pm 0.20 \times 10^6 \text{CFU/g}$, while in the intestine samples is $1.36 \pm 0.27 \times 10^6 \text{CFU/g}$ and in the skin samples it is $0.77 \pm 0.21 \times 10^6 \text{CFU/g}$. All the samples collected showed bacterial growth on Eosin methylene Blue (EMB) and Salmonella-Shigella Agar. The 45 samples gave 135 isolates; 28 (15.3%) *Escherichia coli*, 30 (22.2%) *Staphylococcus aureus*, 34 (25.2%) *Bacillus sp*, 30 (22.2%) *Salmonella sp*, 13 (9.6%) *Pseudomonas auregenosa*. Five isolates were selected characterized by molecular method using 16S rRNA gene sequencing. The antibiotic test of these isolates revealed their sensitivity to ciprofloxacin, pefloxacin, and tarivid. It was indicated that all the bacterial isolate was susceptible to Ciprofloxacin and Sparfloxacin. *Escherichia coli* were also susceptible to Pefloxacin, Ciprofloxacin and Tarivid. *E. coli* was also resistance to Streptomycin, Augmentin and Amoxicillin, Gentamycin, Septrin and Chloramphenicol.

QUALITY, SAFETY AND NUTRITIONAL COMPOSITION OF AQUAPONIC LETTUCE, PAK CHOI, CHERRY TOMATO AND TILAPIA COMPARED WITH MARKET PRODUCTS

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Introduction

Lettuce (*Lactuca sativa*), pak choi (*Brassica rapa* subsp. *chinensis*), and cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) were cultivated in greenhouse aquaponic systems at Sultan Qaboos University. Tilapia (*Oreochromis niloticus*) reared in the system were also analysed. Equivalent market samples were purchased for comparison. Crop and fish samples were analysed for microbiological quality, heavy metals, pesticide residues, and macro- and micronutrient composition. Additional analyses of fish feed, source water, aquaponic water, and hydrostones were conducted to better understand nutrient pathways and possible contamination sources.

Materials and methods

Lettuce (*Lactuca sativa*), pak choi (*Brassica rapa* subsp. *chinensis*) and cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) were cultivated in greenhouse aquaponic systems. Tilapia (*Oreochromis niloticus*) from the aquaponic system were also analysed. Market samples of the same products were purchased for comparison. Crop and fish samples were analysed for microbiological quality, heavy metals, pesticide residues, and macro- and micronutrient composition. Supporting analyses were also conducted on fish feed, source water, aquaponic water and hydrostones to better understand nutrient dynamics and possible contamination pathways within the system.

Results and discussion

Across all vegetable samples, *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes* were not detected, while *Staphylococcus aureus* occurred only in lettuce at very low levels, not associated with toxin formation. Heavy metals were below detection limits and pesticide residues were below the limit of quantification in all crop samples. Nutritional differences were crop-specific. Aquaponic lettuce contained higher magnesium, calcium, potassium, sulphur, and iron, whereas market lettuce contained higher nitrogen, phosphorous and nitrate. In cherry tomato, aquaponic fruits had higher Phosphorous but lower calcium, zinc, and iron than market fruits. Pak choi showed the fewest differences, with only magnesium and total nitrogen differing significantly between sources. Tilapia from both aquaponic and market sources showed a favourable microbiological profile. Supporting analyses further showed that fish feed contained no detectable heavy metals and no quantifiable pesticide residues, while source water and aquaponic water showed no detectable heavy metal or pesticide contamination. Source water also showed a favourable microbiological profile, and hydrostones contained high iron but no detectable arsenic, lead, cadmium or mercury.

Overall, aquaponics produced foods with strong microbiological and chemical safety and nutritional profiles broadly comparable to market products. The findings further suggest that leafy vegetables may be better suited than fruiting crops to low-input coupled aquaponic systems, where nutrient supply may not fully match the higher demands of fruit production. These results support the potential of aquaponics as a sustainable food-production strategy for arid and water-limited regions.

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DIGITAL APPROACH TO OPERATION OF AQUACULTURE INSTALLATIONS

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INTRODUCTION

The so-called “smart” concept of operations is developing to be an increasingly common solution for marine aquaculture. Real time monitoring of asset condition combined with securing a reliable remote operation where the integrity of “autormote” performance is considered a competitive advantage. The fish farming industry is in the middle of a major transition as vessels and installations are transformed into sensor hubs, generating data, and linking in an expanding interconnected web.

DIGITAL APPROACH MERGED WITH REMOTE OPERATIONS

For Smart operations the term digital asset management is often used where inspection as well as operational decisions are based on continuous data streams supplied by sensors on board the fish farm.

Digitally driven condition-based maintenance yields improved reliability by continuously assessing the condition of the fish farming installation. The objective is to identify potential issues before they become major problems and take proactive measures to prevent failures and unplanned as well as expensive repairs. Digital asset management will help improve efficiency and reduce operational costs. For example, by identifying equipment that needs maintenance or replacement. the digitally driven operational monitoring is based on the scheme of capturing information, analysing data collected, learn plus synthesis, and finally act accordingly.

The integrity of these solutions is mainly based on the reliability of sensors and their locations on board. Operational onboard servers together with data transfers solutions and intermediate digital storage are also essential aspects in the utilization of digital management infrastructure. Stability and performance of software applied to process/analyse collected data represents a significant risk and needs to be assessed. Finally safe data transfer and cyber security are vital items in the overall digital performance.

Autonomous and remotely operated fish farms, ranging from remote control operation to fully unmanned installations, marks a major advancement in the aquaculture industry. These solutions can deliver a wide range of benefits including improved personnel safety due to reductions in crew, increased operational efficiency, reduced emissions, and reduced operational and maintenance costs. A broad risk assessment processes for the qualification of autonomous and remotely operated marine technologies with a set of functional requirements need to be established to maintain required safety level for these solutions. This approach is deliberately flexible in nature, and it is essential to creating the space for future innovation, while also drawing on experience from general ocean industry in project development within the autonomous shipping space over the past decade.

While digital asset management can offer several benefits, there are also a number of potential risks and challenges associated with relying on this technology. Together with data security risks and technical issues, challenges related to data quality is a major concern. The monitoring system relies on high-quality, accurate data to provide meaningful insights into asset condition and performance.

This is still early days for autonomous and remotely operated aquaculture installations. Advances in research, technology, and legislation, as well as experience from projects, is expected to lead to significant developments in “autormote” technology in the future

CONCLUSION

Smart-digital is an efficient concept that most operators are chasing, but how well can you trust the status and “green lights” on your operational dashboard? The industry is expected to put great effort into assuring the integrity of the data-streams continuously feeding information to our operational decision-making tools.

There are several efficient methods to mitigate the risks introduced as advanced digital technology is applied in operations of fish farming installations. One of the most rational approaches includes focus on sensor reliability, integrity of operational onboard servers together with robustness of data transfers solutions.

OPERATIONAL CONCEPTS OF NEW-TECH FISH FARMING INSTALLATIONS

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INTRODUCTION

Aquaculture market projections uncover a large supply-demand imbalance in the next three decades. Focusing on fin fish supply specifically uncovers that output level is close to a saturation point for traditional areas with traditional aquaculture. As a result of the current situation fish farming is growing out of sheltered, near-shore areas and moved to more exposed locations and further offshore. Several innovative solutions are being tried out in ocean fish farming in the process of industrialization.

MAIN APPROACH

The numerous concepts planned and implemented may be split into four main categories: Open trusswork with traditional net or grating, semi-closed units, closed aquaculture installation and submerged solutions. All these concepts may be operated with permanent manning or a combination of remote operation and daily visits. This paper supply a specific description of the main solutions applied in the development of ocean farming:

- Open trusswork. Further development of the traditional sheltered net pen usually with rigid floating collar in steel or concrete. Additional structural arrangements for additional buoyancy, ballasting/de-ballasting and distention of net panels are common features. This category may be split into two sub-sets. One includes operating at the ocean surface only while an increasing number of designs have the option of submerged position to avoid splash-zone dynamics at rough sea states.
- Semi-closed units. These designs aim to separate fish environment from sea for the first few meters of the water column. This separation is established to protect fish from parasites living in the upper levels of the water column. Sufficient water circulation to maintain sufficient growing condition and fish welfare is often a change for these solutions.
- Closed fish farming units. A wide variety of solutions are currently being explored in this category. The ability to control quality of water intake to optimize fish environment combined with possibility to collect fish waste and feed surplus promote the applicability of this solution. Water may be cleaned and re-cycled or filtered before emitting to sea resulting in less environmental impact per unit biomass. Establishing full separation between fish environment and sea results in less parasites and disease in addition to absolute fish control (no escape) and controlled aquaculture emissions to marine environment.
- Submerged fish farming pens. An increasing number of designs have the option of submerged position to avoid splash-zone dynamics at rough sea states. This results in added operational complexity as operations such as feeding, dead fish retrieval and similar needs to be conducted remotely from the surface position.

Concepts and solutions listed have several advantages and weaknesses that must be considered and evaluated. In addition to applying steel and concrete as construction materials, several solutions are currently being built in novel materials such as polymers and composites. To reduce risk, utilizing these solutions special focuses on structural integrity and manufacturing quality is needed. This involves a systematic approach across stakeholders to build trust. This work maps out essential items to be considered as qualification of polymer or composite built installations are qualified for safe operations in a demanding marine environment.

The essential parameters to be taken into consideration assessing performance are considered to be: Oxygen level in sea water, crowding and live transfer of fish, dead fish handling and ensilage processing, logistics of supplies and goods going both on and off the fish farming installation and personnel safety.

ASSESSMENT OF GLYPHOSATE AND ITS METABOLITES' RESIDUE CONCENTRATIONS IN CULTURED AFRICAN CATFISH OFFERED FOR SALE IN SELECTED FISH MARKETS IN IBADAN, OYO STATE, NIGERIA

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Introduction

Catfish farming is practiced by around 80% of fish farmers in Nigeria, and it is the most popular variety of fish employed in aquaculture. As a result, the usage of agrochemicals for fish growth and sustainability has increased. On farms, various agrochemicals such as Formalin, Copper Sulphate, Malachite green, and Potassium Permanganate among others have been abused and/or misused (Adeyemo et al., 2011; Alarape et al., 2013). The adverse side effects of glyphosate and aminomethyl phosphonic acid, or metabolites, on soil, water quality, plant, animal, and human health have been studied extensively in recent years due to glyphosate's extensive use and accumulation in the environment and food products (Battaglin et al., 2014). In 2015, the World Health Organization (WHO) categorized the glyphosate herbicide as possibly human carcinogenic based on their most recent findings on its potential chronic negative effects (EFSA, 2015; Guyton et al., 2015).

Materials and Methods

Five adult *Clarias gariepinus* (300 ± 50 g) were collected from each of five active cultured fish markets in Ibadan, giving a total of 25 fish. The fish were pithed, and muscle, liver, and kidney tissues were aseptically harvested, sealed in sterile sample bags, kept on ice, and transported to the International Institute of Tropical Agriculture (IITA) for glyphosate residue analysis. Solid-phase extraction (SPE) and quantification followed Delmonico et al. (2014). Digestion tubes were cleaned with water, distilled water, and 0.5% HCl, then dried. Homogenized tissue samples (0.5 mL) were placed in Pyrex tubes, after which 20 mL of an ammomethyl-phosphonic-nitric acid mixture (5:2:5) was added. Samples were digested for 2.5 hours at 25°C under a condenser, cooled, and diluted to 25 mL with ultrapure water. The mixtures were shaken for 10 min and centrifuged at 5,000 rpm for 5 min. Supernatants were analyzed using Reverse Phase LC (Agilent Technologies) with a C18 column (5 µm, 120 Å, 4.6 × 250 mm) and methanol/water (90:10) as the mobile phase at 1 mL/min for 30 min. The SPE-LC method was validated for specificity, linearity, sensitivity, accuracy, precision, and matrix effect resistance.

Results

The results showed that glyphosate residues were recorded in all the seventy-five (75) fish tissue samples obtained from the selected fish markets in the Ibadan metropolis and all residue concentrations were above both the recommended Acceptable Daily Intake (ADI) of 1.0 mg/kg (1 × 10⁻³ mg/L) and Maximum Residue Limits (MRL) of 0.01 mg/kg (1 × 10⁻⁵ mg/L). Isopropylamine has the highest residue concentration followed by N-Phosphonomethyl and Aminomethylphosphonic Acid (AMPA), while N-Acetyl Glyphosate has the least residue concentration across the sampled markets.

Discussion

The detection of glyphosate residues in the fish organs and muscles in this study confirmed the use of several glyphosate-based herbicides (Force-Up, Vinash, Para force, and Round-Up) on the fish farms (fish as a non-target organism) in Nigeria (Alarape et al., 2024), use of banned pesticides like pyrethroids, organophosphates, carbamates, and organochlorine in agricultural products in Bangladesh (Jallow et al., 2017) and Ghana (Essumang et al., 2009). Although glyphosate-based herbicides were not used directly on fish, environmental contamination may be a possible source of the herbicide residues found in fish in the research location where such herbicides are used on farms close to fish farms. The presence of residues of glyphosate and its metabolites in ready-to-eat fish calls for holistic, systematic, and effective risk management strategies towards monitoring pesticide/herbicide usage in aquaculture production and ensuring the provision of wholesome fish and fish products for the consumers.

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VALORIZATION OF INVASIVE CRAB BIOMASS: THE CASE OF THE CHINESE MITTEN CRAB (*Eriocheir sinensis*) AND ITS POTENTIAL AS FEED INGREDIENT IN AQUACULTURE

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Introduction

Invasive aquatic species are increasingly recognized not only as ecological threats but also as underutilized biomass resources with potential for sustainable valorization (Arena et al., 2024). In this study, we investigated the biochemical composition and biomass characteristics of the highly invasive Chinese Mitten Crab *Eriocheir sinensis* to assess its suitability within a biorefinery framework. Comprehensive analyses were conducted to determine key constituents, including proteins, carbohydrates, and ash content, alongside the potential for enzymatic protein hydrolysis relevant for industrial applications.

Building on this characterization, we explored potential valorization pathways aligned with established biorefinery concepts, aiming to integrate this biomass into circular bioeconomy strategies (Fricke et al., 2023; Fuchs et al., 2025). Particular attention was given to its applicability as a feed ingredient, considering both nutritional value and processing feasibility.

To evaluate this potential, a preliminary feeding trial was conducted using raw, untreated biomass as feed for shrimp *Penaeus (Litopenaeus) vannamei*. Apparent digestibility coefficients were determined to assess nutrient availability and overall feed performance. The results provide initial insights into the digestibility and suitability of this invasive species as an alternative feed resource in aquaculture.

Overall, this work highlights the dual benefit of mitigating ecological impacts of invasive species while simultaneously unlocking their value as a renewable bioresource. The findings contribute to the development of sustainable management strategies that couple environmental remediation with innovative biomass utilization.

Characterization of Biomass

Biomass of CMC was de-shelled and the pure biomass without carapace/chitin was used for biorefinery approaches. Proximate analysis of CMC showed overall good suitability for feeding purposes in aquaculture, with 53.3 ± 0.17 % crude protein.

The biomass was hydrolysed using four different enzymes, resulting in significantly different degree of hydrolysis (DH, Figure 1), as well as different profiles in aminoacids. All hydrolysates showed good levels of anti-oxidant activity revealed by ABTS and TBAR assays. Here, one-way ANOVA revealed a significant effect of enzyme type on ABTS activity ($F(3,8) = 102.43$, $p < 0.001$). Tukey's post-hoc test showed that Alcalase exhibited significantly higher activity than all other enzymes ($p < 0.001$), while Protamex and Flavourzyme did not differ significantly ($p = 0.129$). For FRAP, a significant effect was observed ($F(3,8) = 4.65$, $p = 0.036$). However, Tukey's test indicated that only Protamex differed significantly from Flavourzyme ($p = 0.044$), with no other pairwise differences detected.

Apparent Digestibility in Shrimp *Penaeus vannamei*

The digestibility of biomass from Chinese mitten crab (CMC) were used in a feeding trial with shrimp. We tested raw biomass (with carapace/chitin; CMC+) of *E. sinensis* and de-shelled biomass (w/o carapace/chitin; CMC-) and a control diet as reference (CTRL). Feces were collected over a 5-week timespan, growth and survival were documented. The AD was calculated for crude protein as well as aminoacids and showed suitable digestibility coefficient. All diets resulted similar survival rates (One-way Anova; $F(2,17) = 0.65$; $p = 0.54$) ranging from 92.4 – 97 % and similar hepatosomatic indices (HSI; $F(2,17) = 0.824$; $p = 0.46$) ranging from 3.2 to 3.4, indicating overall good health status during the experimental time. Growth and SGR were highly different between treatments ($F(2,17) = 12.4$; $p < 0.001$), with Tukey's pairwise comparisons revealing significant differences between treatments with CMC- resulting in highest and the CTRL displaying lowest growth. This is a first indication for the suitability of CMC as feed ingredient for shrimp. However, this study was designed as digestibility trial, so this results need to be confirmed with a growth trial, using isoenergetic and isonitrogenous feeds.

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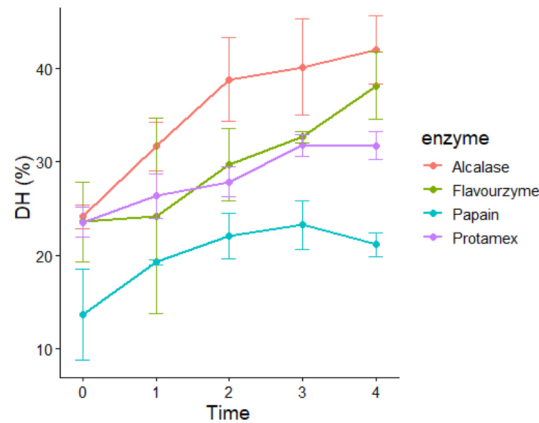


Figure 1 Degree of hydrolysis for four different enzymes used for the protein extraction of Chinese mitten crab biomass

Table 1 Performance data of *P. vannamei* after the 42 day period of the digestibility trial with diets containing either CMC with chitin (CMC+) or without (CMC-) and a control diet (CTRL). Different superscript letters indicated statistical differences (Tukey’s pairwise comparisons, $p \leq 0.05$).

	CTRL	CMC+	CMC-
SGR	1.39 ± 0.22 ^a	1.77 ± 0.17 ^b	2.09 ± 0.31 ^b
Weight gain	9.76 ± 1.57 ^a	12.36 ± 1.21 ^b	14.64 ± 2.18 ^b
survival	0.92 ± 0.11 ^a	0.97 ± 0.07 ^a	0.97 ± 0.05 ^a
HSI	3.28 ± 0.15 ^a	3.24 ± 0.08 ^a	3.43 ± 0.08 ^a

Conclusion

Overall, biomass from the invasive Chinese mitten crab shows suitable nutritional profiles. The biomass can be used as raw biomass, but also shows good potential for biorefinery purposes. Protein hydrolysates of CMC biomass revealed aminoacid-profiles suitable for nutrition in aquaculture as well as good functional properties. A first digestion trial of raw biomass with *P. vannamei* shows its feasibility for the use as aquaculture feeds, but further studies are needed to unfold the whole potential of this underutilized resource.

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PHYSIOLOGICAL IMPACTS OF VALUE ADDED FISH MEALS WITH DIFFERENTIALLY PROCESSED SIDESTREAMS IN ATLANTIC SALMON *Salmo salar* FEEDS DURING ADAPTATION TO COLDWATER AFTER SEAWATER TRANSFER

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Introduction

Atlantic Herring *Clupea harengus* and Blue whiting *Micromesistius poutassou* are two of the most important fish species available for commercial fish meal (Fm) production in Norway. Further, side streams from processing of fish have become increasingly important for fish protein concentrate (FPC) and hydrolysate (FPH) production due to their potential as functional ingredients. Raw material handling and processing of Fm can impact their quality. Traditionally, Fm quality has been determined by raw material freshness and protein digestibility. However, focus on improving the robustness of fish during challenging periods have provided incentive to investigate other avenues to improve the quality of Fm and their side streams. In this study we examined the impact of increasing volatile compounds through gentle processing methods of the raw material and their side streams for improving the physiological robustness in Atlantic salmon smolts during adaptation to seawater transfer combined with lowering of the water temperatures.

Material and Methods

Two qualities of blue whiting Fm namely ‘Superior’ and ‘Standard’ were produced by gentle low temperature and conventional steam drying by Pelagia AS, respectively. Herring Fm of high quality was also produced by Pelagia AS, while the added value ingredients; FPH and FPC were produced from the same batch of fresh, high-quality herring side streams by Nofima AS and Pelagia AS, respectively. Seven nutritional balanced and isoenergetic diets were produced where the control diet was a standard formulated salmon feed supplemented with 15 % high quality blue whiting Fm (D1), standard quality blue whiting Fm (D2) and herring Fm (D3). Iso-protein levels of FPH and FPC (about 4 %) were added to the high quality blue whiting Fm (D4, D5) and to combined 50:50 diets of herring Fm and blue whiting Fm (D6, D7), all balanced to reach 35.5 % water-soluble protein. The nutritional added value potential of the diets was evaluated in a controlled 8-week feeding trial with Atlantic salmon immediately after seawater transfer. The fish were adapted to decreasing temperatures from 12°C in freshwater to respectively 10.5°C (at transfer), 9.5, 8.5, 7.5, and 6.5 °C until final fish sampling.

Results and Discussion

The feeding trial was carried out with salmon (initial weight: 119.5 ± 0.6 g), showing doubling of fish body weight (final weight: 237.5 ± 6.7 g) and efficient growth in all dietary groups (average SGR: 1.23 ± 0.05 %; TGF: 2,60 ± 0.12). No significant differences in feed intake, growth or feed conversion were found in the total feeding period (ns). Increased feed intake, however, was observed in the initial 2 weeks feeding period (10.5°C) in fish fed diets added FPC (D5, D7), while continued increased feed intake was observed only in fish fed the blue whiting diet D5. During the trial, the feed intake slowly decreased in all dietary groups adapting to the steadily declining temperature. Protein and amino acid (AA) digestibility tended to be higher in fish fed D4-D7 added FPH and FPC (Prot: 92.4 ± 0.9 %; AA: 93.5 ± 0.9 %), while no differences relative to the other diets (D1-D3) was found (Prot: 91.3 ± 0.8 %; AA: 92.5 ± 0.7 %). Whole body crude composition (Protein: 16.5 ± 0.2 %; Lipid: 11.8 ± 2.4 %, Ash: 2.10 ± 0.05 %) was unaffected by the diets (ns). The protein retention efficiency was high in all dietary groups, and no significant differences in PER: 3.32 ± 0.14; PPV: 49.5 ± 2.3 % or BV: 53.8 ± 2.4 % was detected (ns). Lipid digestibility was also high in all dietary groups (ADC lipid: 94.3 ± 0.9 %), and no significant differences detected (ns).

In the sampled fish (n=5/tank), the OWI welfare scores of the skin (scale loss, bleeding, wounds and cataract) revealed high quality (OWI scores ≤ 1.2) and no diet dependent differences (ns). Liver lipid (6.4 ± 0.9 %), HSI (1.44 ± 0.10 %), slaughter yield (85.1 ± 0.7 %) and total tocopherols (868 ± 153 mg/kg) were unaffected by diets (ns). The liver contents of Fe and Cu were weakly elevated in fish fed diets added FPC, while liver Zn, Mn and Se were unaffected by diets.

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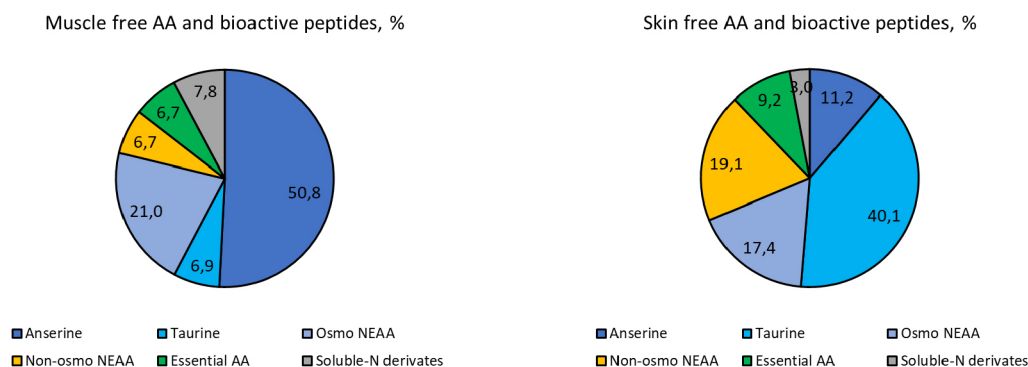


Figure 1 Free amino acids (AA) and bioactive peptides in muscle and skin

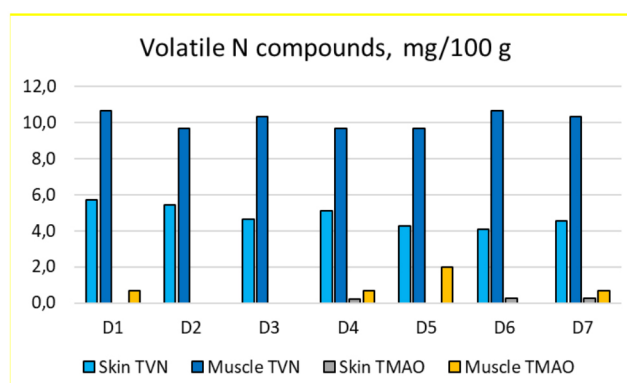


Figure 2 Volatile N-compounds (TVN, TMAO) in muscle and skin

Analyses of free amino acids in muscle and skin showed that nearly 80 and 70 % represented maintenance of the osmotic water balance in the muscle and skin, respectively, independent of the diets, Figure 1. The levels of osmolytes such as anserine was very high in the muscle, while taurine was much higher in the skin, while both appeared to be physiologically well regulated due to the consistent levels and observed differences between skin and muscle of fish from all dietary groups. Addition of the soluble, functional compounds from herring (FPH, FPC) did not seem to affect the tissue composition, while more advanced methods would be required to explore impacts on the physiological regulatory mechanisms.

Summary

Fish from all diets demonstrated efficient growth, feed intake and feed conversion, high protein and lipid digestibility, efficient protein retention and no visual observed differences in skin quality by operational welfare scores (OWI). Distinct differences in the tissue composition of free amino acids and volatile N- compounds in skin and muscle is anticipated to be of physiological relevance for maintenance of osmotic balance but will require further exploring for revealing of the physiological regulatory mechanisms. Additional analyses to study dietary impacts on serum health markers and serum osmolality, amino acid composition in the skin and the muscle, and selected gene expressions in the skin and the gills (qPCR) are in progress and will be presented.

ENVIRONMENTAL DNA-BASED RISK ASSESSMENT OF VIBRIOSIS CAUSED BY *Vibrio harveyi*

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Introduction

Vibrio harveyi is naturally present in marine ecosystems and forms part of the normal intestinal microbiota of many marine organisms. Over the past decades, *V. harveyi* has become an important bacterial pathogen of marine vertebrates and invertebrates, including crustaceans, molluscs, and fish, causing high mortality and substantial economic losses in aquaculture. Its prevalence has increased markedly in Mediterranean aquaculture, likely driven by changing environmental conditions and the effects of climate change. Fish are primarily colonized through exposure to contaminated water, with the bacterium initially establishing on the skin, gills, and gastrointestinal tract. Clinical disease usually develops in immunocompromised fish or under environmental and husbandry-related stress. Biofilms on aquaculture tanks and production systems act as reservoirs of *V. harveyi*, promoting bacterial persistence and increasing tolerance to antimicrobial agents and disinfectants, thereby complicating disease control. Environmental DNA (eDNA)-based monitoring enables the detection of *V. harveyi* before the onset of clinical disease, providing an opportunity for early intervention. Early detection of the pathogen in the environment is therefore essential for timely risk assessment and the implementation of effective disease management strategies.

Materials and Methods

To evaluate the applicability of environmental DNA (eDNA) for early monitoring of *V. harveyi* in marine aquaculture, sampling was conducted at a commercial European seabass (*Dicentrarchus labrax*) farm. Seawater samples (1 L) were collected at two depths: approximately 20 cm below the surface (24.3 °C) and 2 m below the surface (24.0 °C), and filtered through 0.45 µm nitrocellulose membrane filters. Biofilm samples were collected by swabbing a 10 × 2 cm area of the cage pipe, while fouling samples were scraped from the waterline using a sterile knife. Gills and digestive glands were sampled from Mediterranean mussels (*Mytilus galloprovincialis*) that were fastened to plastic cage pipes close to the sea surface. Samples of the kidney, liver, spleen, gills, and heart were taken from four European seabass individuals with clinical indications of vibriosis (uncoordinated swimming). DNA was extracted using commercial extraction kits according to the sample matrix. A species-specific quantitative real-time PCR assay targeting *V. harveyi* was developed using genomic DNA from a reference isolate to generate the standard curve for absolute quantification. Results were normalized to tissue weight (copies g⁻¹), filtered water volume (copies L⁻¹) or sampled surface area (copies cm⁻²).

Results

The developed qPCR assay successfully detected *V. harveyi* DNA in seawater, cage biofilm swabs, mussels and clinically affected European seabass, whereas no amplification was obtained from the fouling sample. Fish tissues showed the highest bacterial loads, with gills containing up to 2.3×10^3 copies g⁻¹, while lower concentrations were detected in the heart, kidney, spleen and liver. Seawater samples collected at two depths were positive for *V. harveyi*, with approximately $2\text{--}3 \times 10^3$ copies L⁻¹. Mussels were also positive, with higher bacterial loads detected in gills than in digestive glands. Biofilm swabs collected from cage pipes contained 30–190 copies cm⁻², indicating the presence of *V. harveyi* on cage surfaces.

Conclusion

Quantitative monitoring of *V. harveyi* in environmental samples, supported by analysis of fish tissues and potential reservoirs, provides a valuable approach for early detection of increased pathogen abundance in mariculture systems. This method may facilitate timely implementation of preventive measures before the onset of clinical disease.

Acknowledgment

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IMPACTS OF ARTIFICIAL LIGHT MANAGEMENT AND CAGE SUBMERGENCE ON THE REPRODUCTIVE PHYSIOLOGY AND WELFARE OF FARMED ATLANTIC COD (*Gadus morhua*)

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Introduction

Since 2018, interest in Norwegian cod (*Gadus morhua*) farming has seen a significant resurgence, driven by the development of 6th to 8th generation domesticated strains selected for enhanced growth and disease resistance. To ensure the long-term sustainability of this renewed industry, it is essential to address a persistent challenge and long-standing biological bottleneck: early sexual maturation and the potential release of fertilized eggs within open-sea cages, as highlighted in recent environmental risk reports. Spawning in cages might results in a possible risk of genetic pollution of wild Atlantic cod populations, while the maturation process itself is energetically demanding, often compromising fish health and growth performance. Continuous light treatment (LL) is a common management strategy used to delay maturation by interrupting the photoperiodic control of the reproductive cycle, a process mediated by melatonin regulation, which is sensitive to prior diurnal light exposure. While LL is widely implemented, its efficacy remains inconsistent. To address this, combining continuous light with cage submergence represents a promising solution to stabilize the light environment and minimize the intensity differential between day and night.

Material and methods

To investigate enhanced maturation control in Atlantic cod, two sequential trials were performed using a single cohort of fish at distinct stages of the production cycle. The first experiment (EP1) was conducted in 2023-2024 with fish ranging from 200 g to 1.5 kg, while the second (EP2) focused on fish between 1.5 and 5 kg. We tested, in triplicate, surface cages exposed to high-intensity continuous light (LL, 2500 W) with or without net/tarpaulin shading, as well as submerged cages positioned at 30 m depth (shielded from strong natural daylight) under either LL (600 W) or natural light (NL) conditions. Stocking densities consisted of 1200 fish per cage in EP1 and 240 to 480 fish in EP2. Prior to transfer to the sea cages, LL-groups were held in indoor tanks under continuous light, while NL-groups followed a natural photoperiod. Bi-monthly sampling was conducted across all experimental groups to monitor growth performance and collect blood and tissue samples. We employed ultrasound imaging alongside hormonal, histological, and molecular analyses (gene expression) to provide a comprehensive assessment of sexual maturation. Additionally, health and welfare were tracked through parasite prevalence monitoring and the implementation of a dedicated welfare scoring system.

Results and discussion

In both EP1 and EP2, the gonadosomatic index (GSI) of the NL-groups rose during winter and spring, culminating in spawning. During EP1, this was dominated by males due to their earlier maturation compared to females. In contrast, LL-groups in EP1 showed a delayed GSI increase whereas those in EP2 maintained GSI levels similar to initial values throughout the experiment, with no spawning observed by the end of the trial despite fish reaching commercial size. Notably, the submerged LL group in EP1 exhibited no significant GSI elevation, with females showing no initiation of vitellogenesis. However, fish kept in NL-submerged cages in EP1 presented an increase in GSI similar to that observed in NL-surface cages. In EP1, plasma levels of sexual hormones, specifically estradiol (E2) and 11-ketotestosterone (11-KT), mirrored the GSI and histological findings; 11-KT increased in males across all NL groups, while an elevation in E2 was noted in females from submerged NL cages. Corresponding analyses for EP2 are currently ongoing.

Regarding fish health, experimental treatments significantly influenced parasite dynamics, with a notably higher prevalence of *Caligus spp.* observed in submerged cages, particularly in LL-treatments across both trials. Preliminary welfare evaluations, adapted from established Atlantic salmon indicators, suggest that cod welfare was generally maintained within acceptable limits; however, some skeletal anomalies, specifically in the cranial and vertebral regions, were noted.

These preliminary observations suggest that the efficacy of maturation control may be dependent on the timing of implementation in the sea cages within the production cycle. While LL treatment resulted in only partial inhibition for the smaller fish during EP1, initial observations from EP2 indicate a more robust suppression in larger individuals. Although further histological and hormonal analyses are required to confirm these trends for EP2, optimizing light management during the production cycle appears to be a promising path for the industry to prioritize somatic growth over early maturation.

Acknowledgment

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RIDGING THE INTENTION-BEHAVIOUR GAP: DRIVERS OF SEAFOOD CONSUMPTION AMONG YOUNG NORWEGIAN CONSUMERS

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Introduction and Methodology

Despite a general desire among 70% of Norwegians to increase their seafood intake (Norges sjømatråd, 2023), actual consumption has declined by approximately 12% over the last decade. This “intention-behaviour gap” is influenced by complex factors including price, sensory aversions, and perceived lack of cooking skills (Heide et al., 2023; Bergsnev, 2025). To identify specific drivers for young adults (aged 18–35), a **Choice-Based Conjoint Analysis (CBCA)** was conducted with a representative sample of **n = 1,000**. Participants completed ten choice tasks evaluating cod filet profiles based on three attributes: product form (natural vs. seasoned), sustainability information (e.g., MSC, National Food Safety Authority), and sensory cues (“Scent of the sea”). The study also explored how early childhood experiences shape adult “Green Trust” and consumption habits (Bergsnev, 2025).

Results and Discussion

The conjoint analysis revealed that **sustainability information** was the most critical factor, with a relative importance of **53.6%**. Products featuring the **MSC label** achieved the highest positive utility (0.305), while guarantees from the Norwegian Food Safety Authority also had a strong positive effect, reflecting high trust in national institutional sources (Gullbrandsen, Vormedal & Larsen, 2022). **Sensory cues** were the second most important attribute (33.5%), where the descriptor “Scent of the sea” significantly increased the likelihood of product choice by triggering positive emotional responses (Bergsnev et al., 2024). Interestingly, **product form** (natural vs. seasoned) had the lowest impact on decision-making (12.9%). Open-ended responses confirmed that while health is a primary motivator, price and sensory barriers remain significant (Carlucci et al., 2015). Furthermore, a strong positive correlation exists between **early-life exposure**—such as fishing trips or helping with cooking—and the development of high-frequency consumption in adulthood. These findings suggest the industry should focus on clear, intuitive sustainability signaling and sensory-focused marketing through digital channels and influencers to reach younger demographics.

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DISSOLVED OXYGEN CONCENTRATIONS IN A COMMERCIAL SCALE SALMON FARM UNDER LOW-FLOW CONDITIONS: FIELD MEASUREMENTS AND MODELLING

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Introduction

To ensure optimal growth and acceptable fish welfare, dissolved oxygen (DO) concentrations in fish cages need to exceed a temperature-dependent threshold (Remen et al. 2016). As the Norwegian salmon aquaculture industry moves towards larger production units, DO levels are likely to become a more significant concern, and climate change can compound this problem through higher water temperatures. DO concentrations within open aquaculture sea cages depend on the dynamical interplay of factors such as the oxygen consumption of individual fish, spatial distribution of fish biomass in the cage, water temperature, ambient current speed and direction and the flow field within the fish farm. Since DO varies spatially between and within cages, it is insufficient to simply measure DO at a single point in order to assess the availability of oxygen for the fish in the farm (Berntsson et al. 2026). In this study, DO was monitored at multiple locations in a commercial salmon farm, and two mathematical models of high and low complexity, respectively, were evaluated as tools to predict oxygen conditions and to assess the risk of hypoxia inside cages.

Materials and methods

The field study was conducted at a commercial salmon farm in Mid-Norway with 8 cages of 50 m diameter (see Figure 1) stocked with biomass over a 6 week period in May-July 2025. DO and temperature was monitored at 5, 10 and 15 m depth inside one cage stocked with biomass near the center of the farm, at two cage locations without biomass and at a reference location outside of the farm. Current speed and direction was monitored using profiling current sensors at one location near the center of the farm and at a reference location outside the farm. Two mathematical models were used to simulate DO conditions over the study period. One model used simple relations between the oxygen consumption of fish as a function of temperature, the expected vertical distribution of the fish, the current speed and the diameter of the cages in order to estimate DO levels inside cages. The other model was based on Alver et al. (2024), and used a gridded 3D representation of the entire farm to simulate spatial DO distribution as a function of advection, diffusion and the spatially distributed oxygen consumption of the fish. The output of the two models was compared to the measured DO values in order to assess the quality of model predictions. A comparison of the two models was made in order to assess the trade-off of model complexity, computational cost and the ability to accurately predict DO conditions and the risk of hypoxia affecting fish welfare.

Table/Figure 1

Exposure	Gd Concentration (ug L-1)	Parent History	Experiments
Basal (control)	0	Non-exposed	Embryotoxicity assay
Low	14	-	-
Medium	157	Exposed	Embryotoxicity assay after 20 days of exposure

Table 1 This table highlights the parental exposure levels of Gadolinium (14 and 157 µg L⁻¹) and the subsequent larval analysis used for risk assessment in marine aquaculture.

(Continued on next page)

Results

The study period included temperatures in the range 7–13°C and currents generally below 0.2 m/s at the reference location. Ambient DO levels were in the range 7.5–11.5 mg/l, while measured DO inside the stocked cage ranged down to below 6 mg/l. The 3D model generally predicted DO levels best when using current measurements from within the farm, with RMS deviation on the order of 1 mg/l and overall bias of about 0.1 mg/l at 5 m. The low and high complexity models were compared for similar conditions, meaning a simulation test case where all other cages were considered empty. The overall trends for DO estimated by the low complexity model were often similar to the overall trends displayed by the complex model when considering a time scale of days. There were differences however, especially when investigating hourly variations. The simplified model seems to be more sensitive than the complex model to variations in current velocity, which may be a result of the formulation of the simplified model. A significant limitation of the simple model was the difficulty of representing effects of neighbouring cages. As illustrated in Figure 1, downstream cages tend to see lower DO levels than upstream ones.

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COLD EXPOSURE DRIVES HEPATIC METABOLIC AND OXIDATIVE ADJUSTMENTS IN *Sparus aurata*: MODULATION BY DIETARY PROTEIN HYDROLYSATES

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Introduction

Temperature is a key environmental factor regulating fish physiology and performance, and sudden decreases in water temperature represent a major challenge for warm-water species such as gilthead seabream (*Sparus aurata*) (Ibarz et al., 2010). Dietary protein hydrolysates (PH), as sources of highly digestible peptides and antioxidant amino acids (AAs), may enhance the capacity of fish to cope with the energetic and oxidative demands imposed by cold stress (Siddik et al., 2021; Wei et al., 2017). However, little is known about how PH from different sources modulate hepatic metabolic adaptations to cold stress. Therefore, this study characterised the metabolic and oxidative responses of juvenile gilthead seabream to low-temperature exposure and evaluated the modulatory effects of dietary PH from different sources.

Methodology

Fish were fed four experimental diets for 88 days at 20 °C: a control diet (CTRL) containing 3% commercial PH (CPSP90), and three other diets in which CPSP90 was replaced with PH derived from insect meal (INSECT), fish by-products (FISH), or swine by-products (SWINE). At the end of the feeding trial, fish were either sampled immediately (non-stressed) or exposed to 15 °C for 5 days before sampling (stressed). Plasma and hepatic metabolites, biomarkers of oxidative status, and antioxidant enzyme activities were assessed.

Results & Discussion

As previously demonstrated, replacing a commercial PH with an alternative PH maintained growth performance and feed efficiency in gilthead seabream (Alves-de-Oliveira et al., 2026). Cold exposure induced marked metabolic changes, characterised by decreased plasma cortisol, lactate, cholesterol, and triglyceride levels, together with increased glucose and non-esterified fatty acid levels, consistent with the mobilisation of endogenous energy substrates to maintain energy homeostasis. Metabolomic analyses further revealed significant shifts in carbohydrate and AA metabolism, with distinct metabolic signatures among dietary treatments. Lower hepatic threonine concentrations were observed in FISH- and SWINE-fed fish, whereas INSECT-fed fish showed higher hepatic cholesterol and triglyceride concentrations.

Cold exposure also remodelled the antioxidant defence system by increasing superoxide dismutase and catalase activities while reducing glutathione peroxidase activity, without increasing hepatic lipid peroxidation (LPO). Dietary PH also modulated these responses, with FISH- and SWINE-fed fish showing lower hepatic LPO than CTRL- and INSECT-fed fish despite the stress challenge.

Overall, the results suggest that selected PH can contribute to enhanced resilience to low-temperature stress through the modulation of energy metabolism and redox homeostasis.

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DEFATTED VERSUS FULL-FAT MEALWORM (*Tenebrio molitor*) MEAL AS FISHMEAL REPLACEMENT: EFFECTS ON RAINBOW TROUT (*Oncorhynchus mykiss*) INTESTINAL HEALTH STATUS

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Introduction

Insect meals, particularly from the yellow mealworm (*Tenebrio molitor*), are increasingly recognized as sustainable alternatives to fishmeal in aquafeeds. To date, most studies have focused on defatted insect meals, which contain between 4–14% lipid content [1]. The industrial defatting process involves a thermal-mechanical fat removal step [2], which improves shelf-life but may reduce environmental sustainability and alter the meal's functional properties [3]. Insect-derived lipids have been reported to modulate intestinal microbiota and barrier function [4], understanding the impact of dietary lipid composition on gut health is critical. This study evaluated the comparative effects of replacing fishmeal with either full-fat (ffTM) and defatted (dTM) mealworm meal on the digestive enzymes activities, oxidative stress responses and intestinal histomorphology 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 (TM): a ffTM (crude protein: 51.9%, crude lipids: 32.8%) and a dTM (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, whole intestines were sampled for digestive enzymes activities, oxidative stress enzymatic and non-enzymatic indicators and semi-quantitative and quantitative histomorphology analyses.

Results

At the end of the feeding trial, rainbow trout showed an eightfold increase in body weight, with no significant differences in zootechnical growth parameters, regardless of the dietary treatments. Nevertheless, fish fed the FFMM and DMM diets exhibited reduced feed intake alongside improved feed efficiency.

No significant dietary impact was observed on the activity of α -amylase and chymotrypsin activities. In contrast fish fed the DMM diet showed higher lipase and trypsin activities and those fed the CTRL diet

No significant dietary impact was observed in intestinal lipid peroxidation (LPO), total glutathione (TG), reduced glutathione (GSH), oxidized glutathione (GSSG), GSH-GSSG ratio, glutathione peroxidase (GPx) and glutathione S-transferase (GST) activities. However, catalase (CAT) and glutathione reductase were increased and reduced, respectively, in the FFMM and DMM groups relatively to the CTRL group.

No significant dietary impact was observed in most semi-quantitative parameters, except for the size and regularity of the enterocyte vacuole, which were slightly enlarged in the FM groups than in the FFMM groups. The quantitative morphometric parameters analyzed in the anterior intestine did not vary significantly among dietary treatments, with no significant dietary impact on the cross section total perimeter, total area, villi area, lumen area, muscular layer area, villi density, villi height, lamina propria width, muscular layer width, stratum compactum width and submucosa width of the anterior intestine. In contrast, villus width and enterocyte height were significantly increased in the DMM groups relative to the CTRL groups, whereas the number of villi was reduced between these treatments. Furthermore, fish fed the FFMM diet had lower villi density than fish fed the CTRL diet.

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Conclusion

Overall, defatted mealworm meal (DMM) increased the villus width and enterocyte height in the anterior intestine and enhanced lipase and trypsin activity compared to fishmeal-based control (CTRL). Despite these morphometric differences, both DMM and FFMM showed a similar impact on intestinal antioxidant activity and redox status. These results suggest that while defatted mealworm meal may be a more suitable direct replacement for fishmeal, the use of full-fat mealworm meal could be considered a viable alternative to reduce the reliance on added vegetable oils in sustainable feed formulations.

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PHYSIOLOGICAL RESPONSES OF RAINBOW TROUT (*Oncorhynchus mykiss*) JUVENILES TO FULL FISHMEAL REPLACEMENT WITH DEFATTED OR FULL-FAT MEALWORM (*Tenebrio molitor*) MEAL: EFFECTS OF THERMAL CHALLENGE

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Introduction

Yellow mealworm (*Tenebrio molitor*) is a prominent sustainable alternative to fishmeal in aquafeeds. However, replacing fishmeal can compromise thermal stress resistance if formulation lack essential nutrients critical for antioxidant defence and membrane integrity [1]. This is particularly relevant for insect meals, which often lack long-chain n-3 polyunsaturated fatty acids [2]. In contrast, mealworm-derived immunostimulants and bioactive compounds can potentially modulate fish immune and oxidative status [3].

This study aimed to characterize the comparative effects of full-fat (ffTM) and defatted (dTM) mealworm meal on the physiological, immunological and inflammatory status of rainbow trout (*Oncorhynchus mykiss*) subjected to a 15-day progressive thermal stress challenge.

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 (TM): a full-fat mealworm meal (ffTM, crude protein: 51.9%, crude lipids: 32.8%) and a defatted mealworm meal (dTM, 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. The diets were tested in triplicate during an initial 8-week growth trial under optimal rearing conditions. Following this, nine homogeneous groups of 7 fish each (159 ± 15 g) were assigned to 120L fiberglass tanks within a recirculating aquaculture system. The thermal challenge was initiated by raising the water temperature from 17 °C to 23 °C over 7 days. During the subsequent 15-day challenge period. Fish were fed 2% of the initial biomass, twice daily, over a. In this 15-day period the temperature was raised in 5-days intervals until reaching 25 °C. At the end of the challenge blood was sampled to isolate the plasma for the innate immune parameters analyses, livers were sampled for oxidative stress enzymatic and non-enzymatic indicators analyses and head-kidneys were sampled for inflammatory status markers expression.

Results

No mortalities or signs of disease were recorded during the thermal trial, and fish showed a mean 30% weight increase. The haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and red blood cell count showed no significant differences between experimental diets. In contrast, haematocrit levels were significantly higher in the CTRL and FFMM groups relatively to the DMM groups. Furthermore, fish fed the FFMM diet also showed higher white blood cell counts (WBC) than fish fed the DMM diet. No significant difference between experimental treatments was observed on glucose and lactate levels, nor on the lysozyme, protease and anti-protease activities. However, fish fed the DMM diet showed a significantly higher peroxidase activity than fish fed the FFMM diet. No significant dietary impact was observed on the hepatic activities of catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST) total glutathione (TG) and lipid peroxidation (LPO) levels. In contrast, the fish groups fed the DMM diet showed significantly higher reduced glutathione (GSH) levels than fish groups fed the CTRL diet, and significantly lower oxidized glutathione (GSSG) levels than fish groups fed the FFMM diet. Ultimately leading to the DMM groups showing a hepatic reduced - oxidised glutathione ratio (GSH/GSSG ratio) significantly higher than the CTRL and FFMM groups. The expressions of *Hsp70*, *tnf- α* , *il-8*, *tgf- β* , and *Casp3* was not affected by dietary composition. In contrast, *il-1 β* was significantly upregulated in DMM group relative to CTRL.

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Conclusion

Under a 15-day thermal stress challenge, rainbow trout fed defatted mealworm meal (DMM) showed higher plasma peroxidase activity, hepatic GSH levels and redox status ((GSH/GSSG)). However, the upregulation of pro-inflammatory cytokines and reduced WBC in the DMM group suggest a complex physiological response. Both ffTM and dTM support high survivability and similar hepatic oxidative protection compared to fishmeal. While defatted mealworm meal (dTM) may offer specific antioxidant advantages, full-fat mealworm meal (ffTM) remains a viable alternative that reduces the requirement for supplementary vegetable oils without compromising the ability of rainbow trout to withstand thermal stress.

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TEACHING AQUACULTURE IN PRACTICE – WITH THE ARC MODEL

Making aquaculture understandable and engaging for the next generation

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This pre-study explores the potential for adapting the Irish educational concept The aquaculture remote classroom (The ARC) to a Swedish context. The ARC is a mobile classroom that, through experiential learning and interactive activities, introduces students to aquaculture, sustainable food production, and the blue economy.

Through study visits in Ireland, interviews with key stakeholders, and pilot implementations with Swedish students in grades 4 and 5, the concept's potential has been tested in a Swedish setting. The results show that students generally have limited knowledge of aquaculture, but that engagement increases rapidly when teaching is hands-on, concrete, and experience-based.

The study demonstrates that the concept has strong alignment with the Swedish primary school curriculum and considerable potential to increase interest in future food systems. At the same time, a clear national adaptation is required, focusing on local conditions, species, and career opportunities.

The presentation will highlight insights from the pilot work, provide concrete examples of teaching approaches, and discuss the opportunities and challenges of developing engaging learning environments focused on sustainable food production in Sweden.

One of the conclusions

The pilot study shows that there is a need to increase knowledge about aquaculture among young people. If Sweden is to develop future sustainable food production and strengthen skills supply within the blue economy, more students need to have the opportunity to engage with these issues early in their education. Strengthening awareness and understanding at an early stage is also important for building social license, as public acceptance and trust are crucial for the long-term development of aquaculture. A Swedish concept inspired by The ARC could form an important part of this effort.

DECISION SUPPORT TOOL FOR CLIMATE-INFORMED SEA LICE MANAGEMENT IN FAROESE SALMON AQUACULTURE – INITIAL MODEL DEVELOPMENT

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Sea lice are currently the main obstacle to increasing production in marine salmon aquaculture across Europe. Treatments for sea lice are also one of the largest contributors to fish mortality and a major factor in the industry's carbon footprint. As ocean temperatures rise due to climate change, sea lice are expected to become an even greater challenge, with faster development rates, increased egg production, and higher infection pressure.

Current models used to guide sea lice management are helpful at the regional level but fall short when applied to individual farms. They are unable to accurately simulate future scenarios that involve changes in climate, farm locations, or production practices, limiting their practical use for producers.

Within the OCCAM project (<https://occamproject.eu/>), Case Study 3 seeks to address these challenges through the development of a Decision support tool (DST) for sea lice management. This tool combines a sea lice population model, already used by the Faroese salmon industry, with a hydrodynamic particle tracking system (FarCoast + LADiM) that will be further integrated into climate models. This enables realistic simulations of how lice larvae move and develop under changing environmental conditions.

The Faroe Islands provide an ideal test case due to the limited number of farms and consistent third-party sea lice data, allowing for accurate model validation.

With this DST, salmon farmers will be able to optimise operations by evaluating farm location, stocking density, production cycle length, and timing—all while accounting for environmental change. In parallel, the tool will support policymakers in setting more effective sea lice thresholds and improving marine spatial planning. This solution offers a practical, science-based approach to controlling sea lice sustainably in a warming world.

The initial model development and our first prototype will be presented.

COMPARATIVE TRANSCRIPTOMIC ANALYSIS OF FAST- AND SLOW-GROWING *Sparus aurata* IDENTIFIES CANDIDATE GROWTH BIOMARKERS

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Introduction

The rapid development of aquaculture has positioned it as a vital component of global food systems. Within the Mediterranean basin, gilthead seabream (*Sparus aurata*) has emerged as a dominant cultured species, reflecting both its commercial importance and adaptability to farming conditions. However, growth variability in gilthead seabream still represents a major challenge for Mediterranean aquaculture, as individuals raised under identical conditions often show large differences in growth rate. This stems from multiple factors, including genetic diversity, where inherent differences in genetic growth potential lead to unequal growth rates. Environmental factors, such as water quality and temperature fluctuations further exacerbate these differences, often favoring certain individuals while suppressing others. This inconsistency reduces overall production efficiency by making synchronized harvesting difficult, as fish reach market size at different times. To address these challenges, in most aquaculture companies periodic grading is commonly practiced, where fish are physically separated based on their size to reduce competition and ensure uniform growth within groups. This method offers a practical short-term solution for managing size variation, but it is still labor-intensive and does not address the underlying molecular factors that influence growth. Moreover, conventional gene expression analyses target only a limited group of candidate genes which may not fully capture the genetic landscape regulating growth.

Materials and Methods

Two independent trials were conducted at EPPO/IPMA facilities, where growth differences were identified at initial sampling with mean weights of 14.0 ± 1.8 g (trial 1) and 14.5 ± 2.6 g (trial 2). Then fish were sorted into slow- and fast-growing based on the average batch weight, with density maintained across all tanks. Fish were maintained at a flow-through system and fed a commercial diet, three times per day, until apparent satiety. Trial 1 lasted 100 summer days, while Trial 2 extended for 284 winter days, reflecting seasonal variations. In the final sampling, liver and intestine (in trial 1 and 2) and muscle (only in trial 2) samples were collected from six fish per condition (fast vs slow) for differential gene expression (DGEs) analysis. Total RNA was extracted with a TRIzol GTM reagent, under manufactures instructions, quantified and quality verified by gel electrophoresis before sending for library construction and mRNA sequencing (Novogene Co., Ltd). To select growth biomarkers in liver and intestine, the DEGs in both trials were compared and only the genes differentially expressed in both trials were selected as hits for validation through RT-qPCR. To select growth biomarkers in muscle, the top 5 up- and down DGEs were selected as hits using data only from trial 2.

Results and Discussion

Results show that fast-growing fish outperformed slow-growing ones, demonstrating better growth potential and physiological performance with mean weights in trial 1 of 139 ± 17 g and 194 ± 16 g and trial 2 of 216 ± 54 g and 591 ± 22 g, for slow and fast-growing, respectively. The combination of both trials' biomarkers selection revealed 11 up (including *cacna1d*, *ccbe1* and *hamp*) and 4 down-regulated genes (including *efna2* and *trim21*) in liver and 1 up (*samd9*) and 2 down-regulated genes (*dhrs12* and *irgc*) in intestine, when comparing fast- with slow-growing fish. The top 5 up-regulated genes in muscle were *tmprss9*, *ctxn3*, *nlrp12*, *dpy30* and *znf608*, and top 5 down-regulated genes were *mfap4*, *dhys*, *mdn1*, *lgals3* and *gpr12*. RT-qPCR validation of some genes confirmed the differential expression between fast- and slow-growing fish, concordant with RNA-seq results. These genes play diverse yet interconnected roles in growth regulation, metabolism, immunity, and muscle function. Their differential expression between fast- and slow-growing fish suggests that they could serve as potential biomarkers for growth profiles in *Sparus aurata*. Differential KEGG pathway enrichment analysis between fast-growing and slow-growing fish revealed tissue-specific metabolic reprogramming associated with growth rate. For liver and intestine, only the pathways that are significant in both trials were analyzed, and in muscle all significant ones. In the liver, fast-growing fish showed significant down-regulation of pathways involved in oxidative phosphorylation, potentially indicating a reduced reliance on hepatic mitochondrial ATP production, and cardiac muscle contraction pathway that reflect a systemic metabolic prioritization away from energetically costly maintenance processes. No up-regulated pathways were observed. In intestine, fast-growing fish showed down-regulation of two metabolic pathways, carbon metabolism and biosynthesis of amino acids. This pattern suggests a reduction in endogenous metabolic

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processing and amino acid synthesis within the intestinal tissue, potentially reflecting a reliance on dietary nutrient uptake and a more efficient utilization of absorbed nutrients to support rapid growth. In muscle, fast-growing fish exhibited a mixed but overall, metabolically enhanced pathway profile. Several central energy and metabolic pathways were up-regulated, including oxidative phosphorylation, the citrate cycle (TCA cycle), carbon metabolism, peroxisome activity, drug metabolism – cytochrome P450, mitophagy animal, and valine, leucine and isoleucine degradation. Together, these changes indicate increased mitochondrial activity, enhanced energy production, and active cellular remodeling to meet the high energetic demands of fast growth. In contrast, the ribosome pathway was downregulated in muscle of fast-growing fish, suggesting a potential reduction in protein synthesis capacity or a shift toward more selective and efficient translational processes during rapid growth. This coordinated inter-tissue regulation likely enhances energy efficiency, supporting rapid somatic growth.

These results reveal distinct molecular signatures associated with growth capacity in gilthead seabream and that the use of RNA-seq proved to be effective for identifying genes and regulatory networks directly related to growth performance. Future work includes expanding validation efforts and conducting additional trials to further test the predictive value of these biomarkers in a real-life setting.

Acknowledgment

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EVALUATING EUROPEAN SEABASS (*Dicentrarchus labrax*) TRANSPORT AND CHALLENGE STRESS THROUGH MOLECULAR BIOMARKERS

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Introduction

Aquaculture has become an essential sector for meeting the growing global demand for high-quality protein, with species such as European seabass playing a key role in Mediterranean production. Despite advances in rearing, fish are particularly vulnerable to physiological stress, disease and routine husbandry practices, such as transportation from hatcheries to nursery facilities or exposure to bacterial and parasitic pathogens, that can result in high mortality and significant economic losses.

Nutritional strategies that enhance resilience during these challenges are therefore of great interest for the industry. Diets enriched with bioactive peptides have the potential to support the immune system, improve stress tolerance, promote growth and survival, making them promising tools for increasing the robustness and productivity of juvenile European seabass. This study investigates the effects of a diet containing bioactive peptides on European seabass juvenile performance under commercial farming conditions after a stress event, through the expression of stress-related genes.

Materials and Methods

The trial was conducted at EPPO/IPMA (Olhão, Portugal) to assess the effect of bioactive peptides on an experimental diet (FORT) and to benchmark them against a SPAROS commercial diet (CTL) on European seabass juveniles. Fish were subjected to transportation (~48h) from a hatchery in Italy to EPPO facilities, where they were reared in duplicate 3m³ outdoor tanks, natural photoperiod, flow-through system with 200% renovation per hour and fed each diet (CTL vs FORT) three times per day *ad libitum*. Fish average weight was 5.3 ± 0.6 g (Day 0), and they were reared until day 45 with sample collection at day 0, 7, 14 and 45. Liver from 8 fish were sampled at day 0 (after arrival/transportation stress) and at day 7 (one week after the stress event).

After this, fish were subjected to an infection challenge with *Photobacterium damsela* subsp. *piscicida* (via intraperitoneal injection). The challenge was performed in triplicate in 30L tanks. A control group that was injected with only Phosphate Buffer Saline (PBS) was added to analyze the effects of the injection itself. Liver samples were collected 24h after the stress. Water quality parameters were kept within optimal ranges for the species, and routine husbandry ensured stable rearing conditions throughout the trial. Mortality was observed until 14 days after challenge.

Relative gene expression of *hsp90*, *nrf2*, *gr1*, *fosl1* *prdx5*, and *cat* was calculated using the efficiency-corrected mathematical model described by Pfaffl. *Prdx5*, *cat* and *gr1* are key enzymatic components of the antioxidant defense system, *nrf2* is a transcription factor that induces these antioxidant genes, *hsp90* is a molecular chaperone that promotes protein folding, stabilization, and protection against cellular stress and *fosl1* an immediate early transcription factor that regulates stress, inflammatory, and cell survival responses through the AP-1 signaling pathway. Expression levels of target genes were normalized against the geometric mean of the reference genes *ef1a* and *rps18*.

Results and Discussion

The results showed that the experimental diet did not affect growth between dietary treatments (CTL vs FORT). Regarding the bacterial infection, fish mortality occurred between days 3 and 7 post-infection, from both dietary conditions, which is the predicted timeline for bacterial infection. Cumulative mortality after challenge was significantly higher on FORT (~28% mortality) when compared to CTL (approximately 15%; *p*-value < 0.05) and both treatments had higher mortality than PBS treatment (100% survival), suggesting that this dietary formulation did not provide a protective effect against bacterial infection.

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Immediately after transport (Day 0), juvenile seabass exhibits an acute stress response, with *fosl1* showing high expression (<0.05) when compared with day 7, indicating that fish were in stress after the transport and recovered after 7 days, as evidenced by the expression of this gene as stress marker. Antioxidant defense genes *gr1*, *cat* and *nrf2* were down-regulated on day 0 when compared to day 7 (p -value <0.05), suggesting a temporary suppression of antioxidant gene transcription during the transport, followed by an up-regulation during recovery. *Prdx5* and *hsp90* expression was not affected.

After the bacterial challenge, gene expression patterns were similar to the effect after transportation, with *fosl1* up-regulation after the stress (injection + infection), suggesting a potential role turning on genes required for tissue remodeling, and structural healing, after stress, and antioxidant defense genes *prdx5*, *cat* and *gr1* being down-regulated after stress, reinforcing the pattern that pathogen challenge in European seabass suppresses oxidative stress gene transcription after a challenge, *hsp90* and *nrf2* expression remained not altered. In addition to the effects of the stressors, dietary treatment also influenced gene expression. Fish fed the FORT diet exhibited consistently lower *gr1* expression and higher *fosl1* expression than fish fed CTL, both before and after bacterial challenge. Considering the higher mortality in FORT diet, these results may suggest that the fish had a reduced capacity to maintain redox homeostasis and suffered a stronger stress response.

Overall, *fosl1* proved to be a reliable marker of acute stress in juvenile European seabass, whereas antioxidant gene expression was down-regulated after the stressors. While the bioactive peptide-rich diet altered the expression of genes involved in redox homeostasis and stress responses, the effects were not associated with improved disease resistance, demanding further investigation into the mechanisms underlying its impact on fish health and survival.

Acknowledgments

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OPTIMIZING EARLY NUTRITION IN SEABASS (*Dicentrarchus labrax*): EFFECTS OF WHEAT AND WHEY HYDROLYSATES ON RELATIVE GENE EXPRESSION

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Introduction

European seabass (*Dicentrarchus labrax*) is one of the most produced species in aquaculture production, mainly in Mediterranean production. Despite advances in hatchery technologies, the larval and post-larval stages remain critical bottlenecks due to their sensitivity to nutritional imbalances, environmental fluctuations, handling stress and particularly during the transition from live prey to inert diets.

Early weaning strategies can reduce dependence on live feeds, but their success depends on the nutritional quality and physical properties of the diets, where advances in feed technology - such as fluidized bed agglomeration - enable the production of stable microdiets with improved nutrient availability that are essential for optimal larval development. In parallel, the use of protein hydrolysates from alternative sources (e.g. wheat and whey) has gained attention for their potential to enhance digestibility, growth, and may also improve resistance to handling stress. This study investigates the effects of early weaning using experimental inert diets on European seabass larvae performance alongside the expression of growth- and stress-related genes.

Materials and Methods

The trial was conducted at EPPO/IPMA (Olhão, Portugal) to assess the effect of early weaning using experimental inert microdiets produced by fluidized bed agglomeration technology containing different hydrolysates, and to benchmark them against a control diet. Three diets were tested: Sparos control diet (CTL), a wheat hydrolysate diet (Wheat) and a diet with whey protein sources (Whey).

Fish were reared in triplicate 300-L cylindroconical tanks under controlled zootechnical conditions and fed *ad libitum* with experimental diets under different conditions. Fish were fed from 11 to 50 days after hatching (DAH), and samples were collected at 12, 22, 30, 40 and 50DAH for analysis.

Water quality parameters were kept within optimal ranges for the species, and routine husbandry ensured stable rearing conditions throughout the trial.

Relative gene expression of growth-related genes (*gh*, *myog*, *igf1*, *pparA* and *tgfB*) and stress-related genes (*cat*, *hsp70* and *sod*) was calculated using the Pfaffl method. Expression levels of target genes were normalized against the geometric mean of the reference genes *fau* and *l13*.

Results and Discussion

The results showed that modifications applied during feed production did not negatively affect the growth performance or physiological status of European seabass larvae. Survival and dry weight (22, 30, 40 and 50 DAH) had no significant differences among treatments throughout the trial.

At the molecular level, most of the analyzed genes involved in growth regulation, including *myog*, *igf1*, *pparA* and *tgfB*, were not significantly affected by dietary treatment. *myog* plays a central role in muscle differentiation, *igf1* is involved in somatic growth and protein synthesis, *pparA* regulates lipid metabolism and energy utilization, and *tgfB* participates in tissue growth and cellular differentiation. The stable expression of these genes suggests that the dietary modifications did not substantially alter the molecular pathways controlling these genes, supporting the zootechnical results. Still, a significantly higher expression of *gh* was observed in CTL (*p*-value < 0.05) in comparison with all experimental diets, suggesting an increase in endocrine growth transcript that was not accompanied by differences in *igf1* expression or by improvements in larval growth. One possible explanation is that the *gh* increase may reflect a physiological regulation or a compensatory endocrine response to subtle metabolic differences among diets rather than actual differences in growth performance.

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Regarding stress-related genes, the expression of *cat*, *hsp70* and *sod* was not influenced by dietary treatment. These genes are commonly used as indicators of oxidative stress and cellular stress responses, with *cat* and *sod* acting as antioxidant enzymes, while *hsp70* is involved in protecting cells from environmental and physiological stress. Their stable expression suggests that the experimental diets did not induce oxidative damage or cellular stress, indicating that the feed processing modifications did not compromise larval welfare or physiological homeostasis. This results further supports the comparable survival and growth observed among treatments.

Overall, the present study demonstrates that the evaluated feed production modifications did not negatively affect the development or physiological status of European seabass larvae and can be applied. The absence of significant differences in survival, growth performance, and the expression of most growth- and stress-related genes indicates that the nutritional quality and functionality of the experimental diets were maintained despite the production changes. These findings support the feasibility of implementing the evaluated feed production modifications in larval aquaculture without compromising larval performance or welfare. Future studies should investigate whether these results are maintained over longer rearing periods and later developmental stages.

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IMPROVED UTILIZATION OF DEAD FISH SILAGE USING THE RAGWORM *Hediste diversicolor*

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Introduction

The increasing growth of the aquaculture industry generates large volumes of organic side streams, creating a need for sustainable strategies that promote nutrient recovery and circular resource use (Turchini et al., 2019, Wang and Olsen, 2023). Fish sludge and dead-fish silage represent nutrient-rich by-products from Atlantic salmon (*Salmo salar*) production that could be valorized through bioconversion by extractive low-trophic organisms. The ragworm *Hediste diversicolor* is a promising candidate for this purpose because of its ability to utilize organic waste substrates and convert them into biomass containing valuable proteins and lipids for aquafeed production (Wang et al., 2019b, Anglade et al., 2023). This study investigated the suitability of two dead-fish silage protein concentrates as feed sources for *H. diversicolor* and compared their performance with fish sludge, a substrate already considered relevant for polychaete production.

Material and methods

A 137-day feeding trial was conducted using three dietary treatments: fish sludge (Sludge), Category 2-derived dead-fish silage (K2) from salmon that died before slaughter (not approved as a feed ingredient), and Category 3-derived dead-fish silage (K3) from salmon slaughter by-products (approved as a feed ingredient). Twelve tanks were used in total, with four replicate tanks assigned to each treatment. The dietary effects on polychaete performance were evaluated by measuring survival and growth. Additionally, diet samples and polychaete biomass were analyzed for total organic matter, elemental composition, amino acid composition, lipid content, and fatty acid composition. In addition, selected contaminants were measured to assess the potential risks of horizontal transfer.

Results and discussion

All diets successfully supported the survival of *H. diversicolor*, with no significant differences observed among treatments. In contrast, growth varied considerably between diets. Polychaetes fed K2 and Sludge exhibited significantly higher final dry weights and specific growth rates (one-way ANOVA, $p < 0.05$) than those fed K3. Although K3 contained the highest levels of dry matter and nitrogen, these characteristics did not result in higher growth, suggesting that nutrient concentration alone was not a reliable predictor of feed performance. Differences in feed texture, availability, acceptance, and actual feed intake may have played an important role in determining growth outcomes.

Diet composition also influenced the nutritional quality of the produced biomass. Polychaetes fed K2 contained the highest total amino acid concentration (one-way ANOVA, $p < 0.05$), suggesting efficient protein deposition. In contrast, Sludge-fed polychaetes showed the highest total lipid content. Significant differences were also observed in fatty acid composition. Biomass produced on K2 and K3 generally contained higher relative proportions of polyunsaturated fatty acids (PUFAs) and n-3 fatty acids than biomass produced on Sludge (one-way ANOVA, $p < 0.05$). Notably, Polychaetes fed K3 exhibited the highest relative DHA content and DHA:EPA ratio, despite their comparatively poor growth performance. These findings highlight a potential trade-off between biomass production and nutritional quality depending on the feed substrate used.

The contaminant analyses provided valuable preliminary insight into feed safety but were insufficient for a comprehensive assessment. Total arsenic concentrations were higher in polychaetes fed K2 and K3 compared with those fed Sludge (one-way ANOVA, $p < 0.05$). However, the absence of data on inorganic arsenic, dioxins, and polychlorinated biphenyls (PCBs) prevented a complete evaluation of potential health and regulatory risks. The results of these analyses are still pending.

Overall, the results demonstrate that both fish sludge and dead-fish silage can serve as suitable substrates for the production of *H. diversicolor* biomass, supporting the conversion of aquaculture side streams into nutritionally valuable resources. Among the tested diets, K2 showed the greatest potential by combining strong growth performance with a favorable amino acid profile. Further research should focus on optimizing feeding strategies, quantifying feed intake and digestibility, and conducting more comprehensive contaminant analyses to evaluate the feasibility of large-scale implementation.

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EVALUATING THE EFFICACY OF BIOREFINERY-DERIVED MICROALGAL INGREDIENTS FROM WASTEWATER FOR JUVENILE BIVALVE FEED

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Introduction

Microalgae are the primary feed source for bivalves throughout their life cycle and remain indispensable for hatchery production (1). However, conventional microalgae production requires a high demand for freshwater, nutrients and energy inputs, contributing substantially to hatcheries' production costs (2). Therefore, more sustainable production systems are needed to ensure a reliable biomass supply. Cultivating microalgae using wastewater, namely agricultural drainwater (excess runoff and percolate discharged from irrigated crop fields), represents a relevant circular bioeconomy approach, enabling nutrient recovery while reducing the need for freshwater (3). Additionally, applying biorefinery processing to this microalgae biomass enables the recovery of microalgal fractions for multiple applications and promotes compound availability, enabling its use as feed ingredients (4). However, information regarding the safety and efficacy of these ingredients for bivalve nutrition remains limited. Therefore, this study evaluated the efficacy of whole microalgal biomass and biorefinery-derived fractions of single- and mixed-species produced in agricultural drainwater as feed ingredients for juvenile *Ruditapes decussatus*.

Material and Methods

Five feeds were formulated for juvenile clams of *Ruditapes decussatus*. The control feed consisted of a pilot microalgae-based formulation for juvenile bivalves (CTRL). Four additional diets were obtained by partially replacing ingredients from the control formulation with microalgae cultivated in agricultural drainwater, either as whole-cell biomass or as biorefinery-derived insoluble fractions (retentate). These experimental formulations included whole cells of *Chlorella* sp. (ChC), *Tetrademus obliquus* retentate (TetR), *Chlorella* sp. retentate (ChR), and a mixed retentate from *Chlorella* sp. and *T. obliquus* (MixR). The biochemical composition (protein, lipid and ash content in % of dry weight) of all diets was determined and is presented as the mean of duplicate analytical runs. Juvenile clams were reared under controlled hatchery conditions for 55 days, with the five dietary treatments using three replicate tanks per treatment. Feeding was performed twice a day. Growth was monitored over time using biometric parameters, such as shell morphometrics (shell length, width and area; fortnightly) and biomass parameters (total wet weight; weekly).

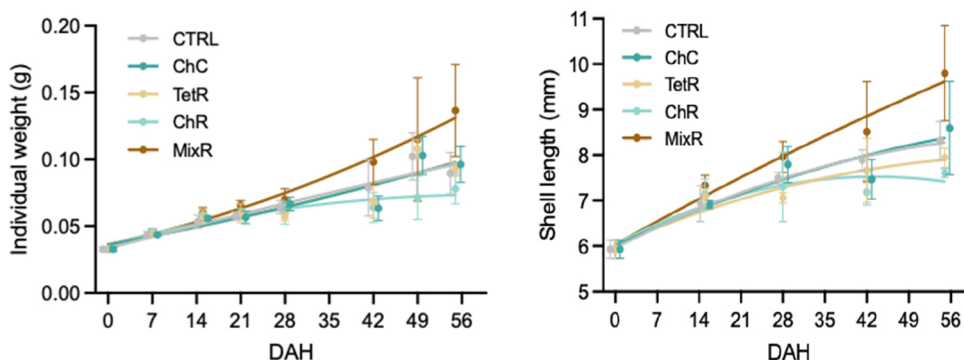


Figure 1 – Individual total fresh weight (g) and shell length (mm) of *Ruditapes decussatus* juveniles over the 55-day feeding trial (DAH). CTRL – control diet; ChC - *Chlorella* sp. biomass; TetR - *Tetrademus* sp. retentate; ChR - *Chlorella* sp. retentate; MixR - mixed *Chlorella* sp. and *Tetrademus* sp. retentate.

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Results and Discussion

Biochemical composition across the five formulations revealed that crude protein content ranged from 20.2% to 26.9%, lipid from 13.7% to 17.6%, and ash from 6.2% to 6.9%. Protein values showed high variability between replicates; therefore, no differences were observed between formulations. The MixR feed presented the highest lipid content (17.6%) compared to all other formulations, which ranged between 13.7% and 15.3%. The highest ash content was observed for the CTRL (6.9%) compared to all other formulations, which ranged between 6.2% and 6.5%. Regarding the feeding trial, all diets successfully supported juvenile growth throughout the 55-day feeding trial. From day 28 onwards, clams fed with the MixR began to present higher individual total wet weight and shell length than the remaining dietary treatments (Figure 1). In contrast, growth of juveniles fed ChC and TetR remained comparable to the CTRL feed, while ChR presented the lowest values at the end of the trial. These observations suggest that combining fractions from different microalgal species may provide additional nutritional benefits compared to single-species fractions. The present results indicate that whole microalgal cells and their biorefinery-derived fractions produced in drainwater can be safely incorporated into juvenile clam diets without compromising performance. This study provides one of the first evaluations of whole microalgal cells and biorefinery-derived fractions produced in agricultural drainwater for juvenile bivalve nutrition, supporting the integration of wastewater valorisation, microalgae biorefinery and bivalve nutrition within a circular production approach.

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INTEGRATING DUCKWEED INTO INTENSIVE CATFISH CULTURE THROUGH CLOSED-LOOP WATER RECYCLING

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The African catfish (*Clarias gariepinus*) is exceptionally well suited to highly intensive inland aquaculture due to its rapid growth, efficient feed conversion, robustness, tolerance of high stocking densities, and facultative air-breathing capacity. These characteristics make it particularly suitable for compact RAS and tank-based systems, enabling high production efficiency with low water requirements. Moreover, *C. gariepinus* can be readily reproduced under indoor conditions, allowing efficient rearing from newly hatched larvae to fingerlings.

Intensive catfish production, however, generates water containing suspended solids and dissolved nutrients, particularly nitrogen and phosphorus. Although RAS substantially reduce water consumption, efficient recovery of dissolved nutrients remains a challenge.

Duckweed, particularly *Lemna* species, grows rapidly in nutrient-rich water and assimilates dissolved nutrients into protein-rich biomass. Under favourable conditions, duckweed can produce 15-30 g dry matter/m² every 1–2 days.

We propose an integrated system combining indoor RAS, outdoor catfish tanks, and duckweed cultivation within a single closed-loop water-recycling system. Water from both fish-production units passes through mechanical filtration before entering shallow duckweed ponds, where dissolved nutrients are assimilated during rapid plant growth. The treated water is subsequently returned to the fish units.

Harvested duckweed is washed, dried, and milled for incorporation into feeds for catfish larvae and fingerlings. Its protein-rich biomass may also provide essential amino acids, vitamins, minerals, and carotenoids.

This approach aims to enhance water reuse, recover nutrients, and produce a local feed supplement, supporting circular and resource-efficient catfish production.

TAURINE SUPPLEMENTATION SUPPORTS IMMUNE AND METABOLIC RESILIENCE TO THERMAL STRESS IN SENEGALESE SOLE *Solea senegalensis* FED PLANT-BASED DIETS

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Introduction

The reduction in the use of marine ingredients in aquafeeds raises concerns regarding the supply of key functional nutrients, such as taurine. This is particularly relevant as plant-derived ingredients, commonly used in fish diet formulations, are virtually devoid of taurine. Taurine plays key roles in osmoregulation, antioxidant defence and immune function, and its deficiency may compromise fish resilience to environmental stressors. Thermal stress is a relevant challenge under climate change scenarios, yet the interaction between taurine nutrition and stress response remains poorly understood. This study evaluated whether taurine supplementation modulates physiological, immune and metabolic responses to thermal stress in Senegalese sole (*Solea senegalensis*) fed plant-based diets.

Material and Methods

Three isonitrogenous and isolipidic diets (59% crude protein and 8% crude fat, dry matter basis) were formulated using practical ingredients. A control diet (CTRL) contained 69% marine ingredients, while in the PLANT diet marine ingredients were reduced to 11%. The PLANTTAU diet was the PLANT formulation supplemented with 1% taurine.

Senegalese sole juveniles (41 ± 1 g) were distributed into 18 tanks in a recirculating aquaculture system and maintained at 19.7 ± 0.5 °C during a four-week conditioning period. Fish were then sampled after 24 h fasting ($n = 3$ tanks per diet). The remaining fish were subjected to a thermal challenge by increasing water temperature by 5 °C for 12 days, followed by a second sampling.

Growth performance, hepatosomatic index (HSI), plasma stress indicators, innate immune parameters and hepatic free amino acids, including metabolites related to methionine metabolism, were analysed.

Results and Discussion

Growth performance and plasma cortisol and glucose were not affected by diet or thermal challenge. However, thermal stress modulated innate immunity, decreasing lysozyme and antiprotease activities while increasing nitric oxide levels.

A significant diet $\times$ stress interaction was observed for peroxidase activity. Post-stress peroxidase activity was highest in CTRL fish, while PLANTTAU reached comparable values, unlike the unsupplemented PLANT diet. This suggests that taurine supplementation supported the maintenance of innate oxidative immune response under thermal stress.

HSI decreased after thermal challenge, suggesting mobilisation of hepatic reserves. Hepatic free amino acid profiles showed marked diet- and stress-dependent changes. The PLANT diet strongly depleted hepatic taurine, whereas supplementation restored taurine levels close to CTRL before stress. Thermal challenge reduced hepatic taurine in CTRL and PLANTTAU fish, suggesting increased taurine utilisation under stress.

Beyond taurine, methionine-related metabolism was also modulated by diet and stress. Hepatic methionine decreased after thermal challenge in all dietary groups, suggesting increased utilisation under stress. CTRL fish showed the highest pre-stress methionine levels and, after stress, a marked increase in homocysteine, cystathionine and cysteine, indicating activation of the methionine–transsulfuration pathway. By contrast, PLANT fish showed lower methionine availability and consistently low homocysteine levels, while cystathionine and cysteine increased after stress. In PLANTTAU fish, methionine was also reduced after stress, but cystathionine and cysteine remained stable, suggesting that taurine supplementation altered the stress-induced adjustment of sulphur amino acid metabolism rather than restoring a CTRL-like profile.

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Diet-dependent responses were also observed for amino acids linked to nitrogen metabolism and redox balance. Glutamine decreased after stress in CTRL, increased in PLANT and remained stable in PLANTTAU. Glycine increased after stress in CTRL and PLANTTAU, whereas it decreased in PLANT. Together, these results suggest that taurine supplementation did not simply restore the overall hepatic amino acid profile of CTRL fish, but contributed to stabilising selected metabolic pools under thermal stress.

Conclusion

Taurine supplementation in plant-based diets for Senegalese sole did not affect growth but modulated selected immune and metabolic responses to thermal stress. While hepatic taurine levels were restored, broader sulphur amino acid metabolism was not fully normalised. Taurine supplementation stabilised specific metabolites and supported post-stress peroxidase activity, highlighting its role in promoting resilience under challenging conditions.

Acknowledgment

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LOW TROPHIC AQUACULTURE AS NATURE BASED SOLUTION: INNOVATIVE APPROACHES FOR ASSESSING MARINE BIODIVERSITY BOOST

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Background

Nature-based solutions (NbS) can be classified according to how much humans intervene and alter ecosystems. These classifications are useful for terrestrial, coastal, and marine environments and aim to balance biodiversity protection, climate change mitigation, and social-ecological advantages. Low trophic aquaculture, such as bivalve aquaculture, is considered a sustainable aquaculture practice that helps maintain biodiversity and supports ecosystem services like food security, may fall under Type-3 NbS (Ghaedi et al., 2026). However, the extent to which systems like bivalve aquaculture boost or sustain biodiversity is not well understood or quantified, and the trade-offs between risks and benefits are still uncertain.

Methodology and aims

The primary objective of this research conducted in EU-funded TRANSEATION project is to evaluate the beneficial impacts of low trophic bivalve aquaculture as NbS on biodiversity in mussel production operations utilizing longline and raft infrastructures, while also considering possible adverse effects on ecosystems. Forty ropes measuring six meters each, seeded with mussels of 4-5 cm shell length (at a density of 400-600 individuals per rope linear meter), were deployed within longline and raft setups located in offshore and port environments, respectively. The study investigates biodiversity changes linked to longline and raft aquaculture by incorporating multi-method observations at multiple stages: before, during, and after production cycles. This methodology integrates both water column and benthic habitat assessments through diverse and complementary techniques. Acoustic data were obtained via an innovative triple-frequency acoustic buoy installed on both the longline system and mussel raft, permitting ongoing monitoring of the water column throughout 1-year cultivation period. These acoustic findings are augmented by detailed image analysis and direct sampling of the benthic habitat, furnishing a holistic understanding of biodiversity dynamics within ecosystem compartments. A comprehensive rating system integrating these datasets was developed to appraise the ecological effectiveness of these infrastructures as NbS, taking into account the temporal and spatial dimensions of mussel production.

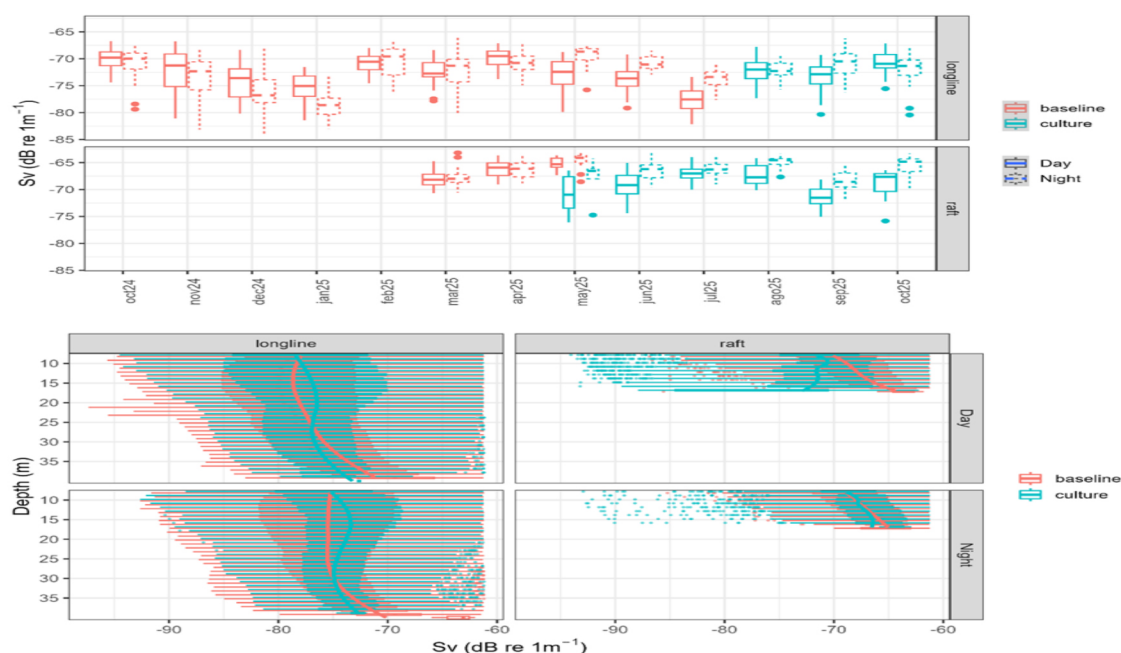


Figure 1. Fish abundance temporal and depth variations associated to mussel longline and raft culture areas. The study demonstrates a scalable methodology for assessing ecological impacts and benefits of aquaculture practices, supporting more sustainable management and improved evaluation of NbS performance in marine systems.

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This analysis aims to identify opportunities for expanding bivalve aquaculture by leveraging its environmental benefits—such as supporting biodiversity conservation—as NbS, while considering both the challenges and advantages involved in promoting sustainable aquaculture practices.

Results and conclusions

Preliminary findings indicate marked seasonal fluctuations in acoustic fish abundance at both aquaculture sites, with consistently greater values observed at the raft compared to the longline system (Figure 1). At the longline location, fish abundance reached its highest levels during autumn and spring and showed reductions in winter and summer. The shorter time series at the raft site similarly demonstrated seasonal variation, notably a distinct decline during the onset of cultivation and peak abundance in summer. Initiation of farming corresponded with significant alterations in vertical distribution: at the longline, maximum abundances transitioned from deeper strata prior to cultivation to surface layers during both daytime and nighttime post-cultivation; at the shallower raft site, changes were primarily apparent during daylight hours, with substantial decreases in bottom-layer abundances following the commencement of farming activities (Figure 1). Benthic surveys were undertaken within the culture areas, and benthos samples together with ROV imagery are currently being processed for taxonomic assessment of the benthic communities. Final results from this study are anticipated to be available in July 2026.

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VALORIZATION OF THE INVASIVE ALGAE *Rugulopteryx okamurae* THROUGH ITS USE IN SEA URCHIN (*Paracentrotus lividus*) FEEDING

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Introduction and methods

Sea urchin aquaculture, particularly *Paracentrotus lividus*, is gaining increasing interest due to the high market value of its gonads and the need to reduce fishing pressure on wild populations. At the same time, the invasive seaweed *Rugulopteryx okamurae* has become an ecological and economic concern along the Portuguese coast, affecting biodiversity, fisheries and tourism (Boesiger, 2024). However, its high biomass availability may represent an opportunity for valorization within a circular blue economy framework. This study evaluated the use of *R. okamurae* as a feed source for *P. lividus*, either as a dried macroalgal diet or incorporated into formulated feeds. Four diets were tested: dried *Ulva* sp., dried *R. okamurae*, a formulated diet containing 20% *Ulva* sp., and a formulated diet containing 10% *Ulva* sp. + 10% *R. okamurae*. *Ulva* biomass was produced at IPMA facilities and solar-dried in greenhouse trays, while *R. okamurae* was harvested and dried by Easy Harvest Lda. Formulated diets were produced by Sparos I&D using a previously validated formulation for *P. lividus* (Araújo et al., 2023). The trial lasted 139 days and was conducted in open-circuit systems under natural environmental conditions, monitored on a daily basis. Diet effects were assessed in juveniles through survival, feed conversion ratio and growth performance, and in pre-commercial sea urchins through gonadosomatic index (GSI) and gonad proximate composition. Sea urchins were fed every two days, with uneaten feed recorded before each feeding. Weight and test diameter were measured monthly. Differences among treatments were analyzed using one-way ANOVA or Kruskal–Wallis tests, followed by post-hoc comparisons.

Results and discussion

The results of this study demonstrate that the invasive macroalga *R. okamurae* can be incorporated into the diet of sea urchins (*P. lividus*), although its effectiveness is highly dependent on the mode of inclusion. Sea urchins fed exclusively with dried *R. okamurae* exhibited poor zootechnical performance, including weight loss, minimal test growth and a markedly reduced gonadosomatic index (GSI), indicating limited nutritional suitability as a sole feed source (see table1). These findings are consistent with the presence of secondary metabolites, such as diterpenes, which are known to reduce palatability and digestibility (Casal-Porras, 2021). Nevertheless, continuous ingestion and high survival rates (>94%)

Table 1: *Paracentrotus lividus* production parameter: Total Diameter Gain (TDG), Total Specific Growth Rate for Diameter (TSGRD), Feed Conversion Rate (FCR) and gonads proximal analysis

sea urchins production parameters	Diet	TDG (%)	TSGRD (% day ⁻¹)	FCR
	Dry <i>Ulva</i> spp.	9.01 ± 6.33 ^{ab}	0.06 ± 0.04 ^{ab}	11.17 ± 1.87 ^a
	Dry <i>R. okamurae</i>	0.97 ± 1.08 ^b	0.01 ± 0.01 ^b	n.a.
	<i>Ulva</i> -based Feed	13.27 ± 2.72 ^a	0.09 ± 0.02 ^a	6.5 ± 0.96 ^a
	Mix Feed	11 ± 2.88 ^a	0.07 ± 0.02 ^a	7.58 ± 0.98 ^a
Sea urchins gonads proximal analysis	Diet	Protein (% DM)	Fat (% DM)	Energy (MJ/kg DM)
	Dry <i>Ulva</i>	60.10 ± 4.38	10.11 ± 1.28	20.19 ± 0.53
	Dry <i>R. okamurae</i>	55.54	10.13	20.77
	Mix Feed	54.77 ± 5.77	13.29 ± 0.95	21.38 ± 0.68
	<i>Ulva</i> -based Feed	55.32 ± 1.29	13.44 ± 0.70	21.53 ± 0.20

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suggest the absence of acute toxic effects, highlighting the trophic plasticity of the species and its capacity to adapt to novel food sources. In contrast, the inclusion of *R. okamurai* in a formulated diet resulted in significantly improved performance, yielding the highest somatic growth and GSI values among treatments, surpassing both macroalgal and control feed diets. This indicates that processing and dietary balancing can mitigate the limitations associated with raw biomass, enhancing nutrient availability and feed efficiency. Proximate analysis of the gonads revealed that dietary treatments influenced their biochemical composition, reflecting differences in nutrient assimilation and allocation. In particular, formulated diets promoted a more favourable nutritional profile, consistent with enhanced gonadal development and improved production performance. Overall, the results support the potential valorization of *R. okamurai* as a functional ingredient in aquafeeds, contributing to circular economy strategies by transforming an abundant invasive species into a valuable resource. However, further research is required to optimize inclusion levels, assess long-term effects and evaluate the sensory attributes and market acceptance of the produced gonads, which remain critical for commercial application.

Acknowledgment

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DISTRIBUTION AND MOLECULAR CONFIRMATION OF *Perkinsus mediterraneus* IN EUROPEAN FLAT OYSTERS (*Ostrea edulis*) FROM ITALY AND CROATIA

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Introduction and Methods

The European flat oyster (*Ostrea edulis*) is a key species for sustainable aquaculture and restoration programmes in the Adriatic Sea. However, information on emerging pathogens affecting this species remains scarce. Within the Interreg Italy–Croatia MARINET project, a cross-border health surveillance programme was carried out to investigate the occurrence of listed and emerging pathogens in farmed *O. edulis* from Italy and Croatia. Oysters were collected between 2024 and 2026 from seven farming areas located along the Italian (Duino, Cattolica, Mattinata and Gallipoli) and Croatian (Lim Bay, Budava and Mali Ston Bay) coasts. Samples were examined using histology, cytology and duplex real-time polymerase chain reaction (PCR) assays for listed diseases (IFREMER). Specimens showing histological features compatible with *Perkinsus* infection were further analysed by end-point PCR (Casas *et al.*, 2002), sequencing and phylogenetic analysis to confirm species identity.

Results and Discussion

No infections with *Marteilia refringens* or *Bonamia ostreae* were detected in both Italian and Croatian farms. In contrast, *B. exitiosa* was detected exclusively in Italian oyster farms, with 11 positive oysters in Duino, one positive oyster in Mattinata and two positive oysters in Gallipoli, whereas all Croatian farming sites (Lim Bay, Budava and Mali Ston Bay) and Cattolica tested negative.

Perkinsus-like organisms were detected in oysters from all investigated areas, although prevalence varied among sites. Positive oysters were recorded in Duino (52/330), Cattolica (5/30), Mattinata (1/20), Gallipoli (6/20, increasing to 12/30 during a mortality event), Lim Bay (8/120), Budava (7/123) and Mali Ston Bay (3/65). Histological examination revealed multiple developmental stages in connective tissues of the visceral mass, mantle, gills, labial palps and gonads, frequently associated with haemocytic infiltration. Molecular characterization confirmed all analysed isolates as *Perkinsus mediterraneus*. In Italian sites, parasite occurrence appeared to be influenced by temperature, with higher prevalence observed at intermediate water temperatures (approximately 17 °C). Overall, infections occurred at low to moderate prevalence, suggesting an endemic distribution.

This study represents the first confirmed report of *P. mediterraneus* in the Adriatic Sea, extending the known geographical distribution of the parasite and highlighting the value of integrated histological and molecular surveillance for detecting emerging pathogens in *O. edulis*.

Acknowledgment

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EVIDENCE FOR IMMUNE PRIMING AND CROSS-PROTECTION IN *Magallana gigas* AGAINST *Vibrio splendidus*

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Introduction

Bacterial pathogens of the genus *Vibrio* represent a major challenge in oyster aquaculture and are associated with substantial mortality events worldwide. Immune priming has emerged as a potential mechanism by which invertebrates can enhance protection following prior pathogen exposure. However, the specificity and breadth of such responses remain incompletely understood in oysters.

Materials and Methods

Pacific oysters (*Magallana gigas*) were exposed to sublethal doses of *Vibrio splendidus* strains differing in origin and virulence characteristics. Humoral immune responses were evaluated using cell-free haemolymph plasma and bacterial growth assays under controlled conditions.

Results and Discussion

Exposure to sublethal bacterial concentrations resulted in inhibitory effects during subsequent challenge conditions. Stronger responses were observed against sympatric strains, while partial cross-protection against allopatric strains was also detected. Differences between in vitro and in vivo observations suggest that humoral immune components may contribute substantially to immune priming effects.

Conclusion

Understanding immune priming mechanisms in oysters may provide insights for disease mitigation and sustainable aquaculture practices. These findings highlight the importance of host–pathogen interactions and may contribute to improved management strategies for shellfish production.

EFFECT OF ALTERNATIVE DIETS ON CAROTENOID CONTENT, COLOUR PARAMETERS AND BIOACCESSIBILITY IN SEA URCHIN *Paracentrotus lividus* GONADS

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Introduction

The sea urchin *Paracentrotus lividus* is an echinoderm widely distributed along the coasts of the Mediterranean Sea and the eastern Atlantic Ocean, extending northward to Ireland. This species is harvested for its gonads (roe), which are highly valued as a seafood delicacy in many European cuisines. However, increasing demand for roe, combined with overfishing, climate change, and pollution, has led to a progressive decline in natural stocks over recent decades. In response, several countries, including Italy, have implemented management measures for sea urchin fisheries at both national and regional levels. In this context, the development of echinoculture represents a promising strategy to mitigate overexploitation through restocking initiatives, while also providing a sustainable supply of biomass for the market. To advance knowledge on diet formulation and its suitability for echinoculture - currently the main constraint limiting the development of this practice, this study investigates the effects of experimental feeding regimes. Specifically, the green algae *Ulva* spp. (class Ulvophyceae) was selected as the primary substrate due to its sustainability, low cost, and wide availability in many coastal lagoons (Addis et al., 2023). In addition, meal derived from the black soldier fly, *Hermetia illucens*, was included as an alternative protein source; insect-based meals have recently been authorized in Europe as feed ingredients in aquaculture (EPRS, 2024).

The aim of this study is to evaluate the effects of two experimental diets on gonadal growth and quality, assessed in terms of color, carotenoid profile, and bioaccessibility.

Material and Methods

Trials were carried out at the research facility GillaLab (University of Cagliari, [Home - GillaLab](#)). Sea urchins (n= 240) were fed with two experimental pellet diets 1) the green seaweed *Ulva* spp. (UL) and 2) *Ulva* spp. supplemented with *Hermetia illucens* meal (IN). These treatments were compared with control groups: sea urchins reared in land-based tanks and fed with fresh biomass (CT), wild sea urchins (SI), and sea urchins reared in open-sea cages (SS). Gonadal growth was assessed using the gonadosomatic index (GSI), while gonad color was measured using a CIELAB colorimetric system L^* =lightness, a^* =red/green and b^* = yellow/blue (Chroma meter CR-410, Konica Minolta Sensing, Inc., Tokyo, Japan). Carotenoid profiles were determined by chromatographic analysis and *in vitro* digestion (INFOGEST 2.0 protocol) was applied to assess carotenoid bioaccessibility (Brodkorb et al. 2019). The experiment lasted 30 days.

SS	CT	UL	IN	SI
CIE $L^*a^*b^*$ (mean)	CIE $L^*a^*b^*$ (mean)	CIE $L^*a^*b^*$ (mean)	CIE $L^*a^*b^*$ (mean)	CIE $L^*a^*b^*$ (mean)
$L^* = 81.93$	$L^* = 80.95$	$L^* = 79.18$	$L^* = 78.27$	$L^* = 77.74$
$a^* = 7.48$	$a^* = 6.99$	$a^* = 10.70$	$a^* = 11.99$	$a^* = 10.50$
$b^* = 27.72$	$b^* = 24.81$	$b^* = 25.72$	$b^* = 24.39$	$b^* = 22.47$
RGB (247, 212, 183)	RGB (250, 208, 169)	RGB (232, 188, 149)	RGB (231, 185, 149)	RGB (219, 169, 128)
HEX #F7D4B7	HEX #FAD0A9	HEX #E8BC95	HEX #E7B995	HEX #D8A980

Figure 1. Colour model of gonad samples from the different experimental groups (CT, UL, IN, SS and SI). L^* , a^* , b^* values, as well as RGB and HEX codes, are reported.

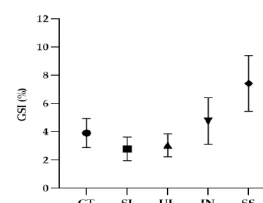


Figure 2. Gonad-somatic index (GSI, %) of (CT, UL, IN, SS and SI). Data are expressed as mean $\pm$ standard deviation.

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Results and Discussion

The highest GSI values were observed in SS (7.42 ± 1.97) and IN (4.77 ± 1.65), followed by CT (3.91 ± 1.03), UL (3.04 ± 0.81), and SI (2.79 ± 0.84). The ΔE^*ab index represents the comprehensive color difference between two samples in the CIELAB color space. Color variation among gonad samples were determined with SS as the reference. Gonads from UL and IN groups showed color parameters comparable to wild specimens, with low chromatic differences ($\Delta E^*ab = 6.70$ and 7.20 , respectively), indicating acceptable visual quality. Carotenoid analysis revealed lutein, echinenone, and amauroxanthin as the main compounds, with significant variability among treatments. Higher lutein concentrations were found in the CT ($10.93 \pm 0.65 \mu\text{g/g}$) and SS ($8.29 \pm 0.38 \mu\text{g/g}$), while UL and IN, showed the lower levels (4.44 ± 0.28 and $4.07 \pm 0.24 \mu\text{g/g}$, respectively).

The INFOGEST 2.0 protocol highlighted how the bioaccessibility values (%) of IN sea urchin for lutein and echinenone were generally higher compared to those observed in UL and CT.

Overall, although experimental diets resulted in lower carotenoid content, they produced gonads with colour characteristics similar to wild individuals, suggesting their potential suitability for echinoculture. The inclusion of insect meals improved gonadal growth, highlighting a promising strategy for sustainable feed formulation.

Acknowledgment

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OPTIMIZING AQUACULTURE BREEDING PROGRAMS WITH EARLY-LIFE GENOTYPING

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Aquaculture breeding programs continue to evolve as genetic tools become more accessible and integrated into routine practice. Greater availability of reliable genotyping tools has been crucial for enabling genetic progress in breeding programs.

This talk explores how genotyping candidate broodstock early in life within a genomic selection framework can improve breeding outcomes. This approach is widely used in terrestrial livestock breeding programs, such as cattle and swine, but is less commonly applied in aquaculture programs. Using insights from early-life genotyping provides a powerful framework for making earlier and more informed breeding decisions. This approach is particularly valuable in species with longer production cycles, where the cost of raising animals to maturity is high. In this framework, only a small number of high-value candidate broodstock need to be raised to maturity, resulting in greatly reduced costs for feed, facilities, and personnel.

Early-life genotyping enables breeders to assess genetic merit as soon as individuals are large enough to individually tag, improving selection accuracy and intensity while reducing infrastructure requirements and costs. Early genotyping also helps maintain genetic diversity by enabling balanced representation across families, avoiding the unintended loss of valuable genetic variation that can occur with traditional phenotype-based culling strategies. Selective reductions over the course of the life cycle allow for phenotypes to be assessed at multiple time points, decoupling early-life phenotypes from late-life phenotypes, and can result in genetic improvements to production traits that would not be possible from late-growth alone. Importantly, even when unfavourable genetic correlations exist between key traits, the added precision provided by the data allows breeders to make more balanced and informed decisions.

Case examples from commercial breeding programs demonstrate how integrating early-life genotyping into selection strategies can improve key production traits. This strategy not only improves the efficiency and speed of genetic gain but also changes how selection decisions are made across the breeding cycle for more cost-effective aquaculture breeding programs.

MASSIVE OPEN ONLINE COURSE (MOOC) IN SUSTAINABLE AQUACULTURE FOR LOW TROPHIC SPECIES

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The expectations for sustainable food production in aquatic systems are high, especially of low trophic species products with a low carbon footprint. To enhance the knowledge among students, practitioners, and teachers, we developed a massive open online course (MOOC) in sustainable aquaculture for low trophic species (SALTS). The course was launched in April 2023 and contributes to the much-needed increased literacy in the field necessary for enhanced and diversified aquaculture production. The course is free, easily accessible and students may progress through the modules at own pace. By now more than 1000 individuals have signed in to the course and over 100 have completed the course and received the certificate. In this presentation, we describe the methodological approach for MOOC development, present the course content and structure, as well as student evaluations and recent updates of the course.

How to develop a MOOC

According to the constructive alignment theory, deciding on learning outcomes is the first and the most important step in developing a course. Learning outcomes will guide what material should go into the course and what type of assessments will be used. SALTS is a master level course, which means we expect the students to acquire higher levels of cognitive learning (Revised Bloom's Taxonomy – Apply, Analyse, Evaluate, and Create). Higher levels of learning expect students to have a good knowledge of the concepts. Therefore, some learning activities and assessments must occur at the lower levels of cognitive learning (Remember, Understand). The aim is to cover the value chain of several low trophic species through 10 modules. The AquaVitae Horizon 2020 project scientists provide the research-based content needed, and some of them have contributed to the course updates.

Course modules and the pilot

The modules are: 1) Sustainability of Low Trophic Aquaculture (LTA), 2) LTA Governance, 3) Food nutrition and safety, and consumer perspectives 4) Business, economics, and markets, 5) Production systems, 6) Macroalgae, 7) Echinoderms, 8) Molluscs, 9) Herbivorous finfish, and 10) Future of LTA. Each module consists of a short introduction video followed by an engagement activity. The module contains 2-3 sub-topics, including videos, quizzes, reading material, advanced assessment and additional material. This educational material has been provided for online students, and for teachers who can utilise the material in a flipped classroom format. The entire course is equivalent to 5 ECTS points (125-140 hours in total). Each module takes approximately 13 hours to complete. The course is available on the Open edEX platform: Sustainable Aquaculture for Low Trophic Species | UiT OpenedX.

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ECONOMIC LOSSES FROM DISEASE IN GLOBAL AQUACULTURE

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Disease is a major challenge that limits aquaculture production and productivity globally. Intensification of aquaculture has led to enhanced risk of disease emergence, although the increased control with the production process enhances the ability to prevent or handle disease. While disease management requires significant investment in disease control, the largest cost associated with disease is most often foregone revenue even when extreme preventative and treatment costs are incurred. However, despite the significant challenges associated with disease, the losses associated with disease in aquaculture are poorly documented, and there are surprisingly few recent estimates despite the continued rapid increase in aquaculture production. The FAO estimated losses to the global aquaculture industry were \$6 billion per year over a decade ago, and more recent studies quantifying the economic costs typically focus on specific diseases, species, or regions, precluding a comprehensive understanding of the magnitude of disease at a global scale and how the challenges differ by species. Most published studies also document losses in wealthy countries despite these countries contributing a relatively small share of global aquaculture production.

In this paper we will provide estimates of disease cost in aquaculture globally, as well as by species and country. We estimate the global production losses to be \$16.0 billion per year or 5.1% of the global production value. The losses rates vary significantly by species, with shrimp having the highest loss rate with 29.6%. With its high production value it then makes up about one quarter of the total disease cost in aquaculture.

INTEGRATIVE OMICS ANALYSIS IDENTIFIES CANDIDATE CAUSATIVE MUTATION FOR RESISTANCE TO CARDIOMYOPATHY SYNDROME IN ATLANTIC SALMON

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Introduction

Cardiomyopathy syndrome (CMS) is a severe viral cardiac disease of Atlantic salmon caused by piscine myocarditis virus (PMCV). The disease typically manifests several months after sea transfer, during the second year of production when fish reach approximately 3.6 kg. Clinical signs are often absent until mortality begins, resulting in compromised fish health and welfare as well as substantial economic losses for producers. CMS is characterized by a slow progression of pathology, and even minor stressors, such as treatment for sea lice infestation, may trigger mass mortality events [1]. Histopathological examination reveals severe inflammation and necrosis primarily affecting the endocardium and spongy myocardium of the atrium and ventricle [2], accompanied by extensive infiltration of mononuclear immune cells, including lymphocytes and macrophages [3]. Estimates of heritability for resistance to CMS vary widely depending on phenotypic definitions (e.g., survival, viral load, or histology), environmental conditions (field versus controlled challenge), and population structure. Previous studies have identified genomic regions on chromosomes 12, 23 and 27 associated with genetic variation in CMS resistance, with the major quantitative trait locus (QTL) on chromosome 27 explaining approximately 30% of the genetic variance [4]. In the present study, we aimed to fine-map these genomic regions, with primary focus on the QTL located on chromosome 27, and to identify causative genes and/or mutations underlying host resistance to CMS.

Materials and Methods

The study population originated from the MOWI ASA breeding nucleus and included four year classes (YC2016, YC2017, YC2019, YC2021). Individuals were selected for whole-genome resequencing (WGR) to fine-map previously identified QTL regions and for functional validation using transcriptomic (atrium) and proteomic (plasma) analyses. WGR was conducted on YC2016–YC2019, while YC2021 was used for expression analyses. All individuals had pedigree data, CMS phenotypes (challenge or field), and genotypes from the NOFSAL03 (~55K SNPs) array. Sampling was stratified by genomic breeding values (gEBV) and haplotype status, limiting relatedness (≤ 4 per sire, ≤ 2 per dam). Individuals were classified into three groups resistant (FUL; gEBV > 1.25 SD, two favorable haplotypes), intermediate (HET; > 0.625 SD, one copy), or susceptible (DEL; < 1.25 SD, no copies). A total of 157 individuals were selected for WGR and 30 ($n=10$ per group) from YC2021 for transcriptomic and proteomic analyses. Sequencing (150 bp paired-end) was performed on the BGISEQ platform. Reads were quality-filtered (using Trimmomatic), aligned to *ICSASG_v2* (using BWA-MEM), and variants called using SAMtools/bcftools, and variant annotations via Ensembl VEP. Transcriptomic data were generated on Illumina NovaSeq 6000, processed with STAR, and analysed with edgeR. Plasma proteomics was conducted using Q-Exactive Orbitrap, with analysis in MaxQuant and statistical evaluation in Perseus and Unscrambler. Genome-wide association analysis using sequence-derived variants was performed using linear mixed model in GCTA [5] with “--mlma-loco” function, including year class as a fixed effect and additive genetic effects based on a genomic relationship matrix: $y = \mu + \alpha + e$, where y is the vector of phenotypic records, μ is the overall mean, and α represents fixed effects with corresponding design matrix X . The vector α denotes additive genetic effects, with G as the genomic relationship matrix and design matrix X , while e represents residual effects.

Results and Discussion

Alignment of sequence reads within the target QTL region on chromosome 27 (10–12 Mbp) identified 8,813 variants, including five high-impact SNPs spanning approximately 13 genes. Among these, two immune-related genes, *MRI* and *HAI1F*, emerged as strong candidates. Genome-wide association analysis reinforced a robust signal in this region; however, inclusion of sequence-derived variants did not further increase statistical significance beyond that captured by the SNP array. Moreover, high-depth (~100×) resequencing comparing FUL and DEL groups revealed reduced or absent read alignments in specific segments of the susceptible DEL group relative to resistant FUL individuals, indicating the presence of a structural variant, most likely a deletion spanning exonic regions of the *HAI1F* gene. This putative deletion was experimentally validated at both genomic and transcriptomic levels using a targeted PCR assay on 30 individuals (10/group), which showed complete absence of amplification in DEL individuals. Additionally, differential expression analysis

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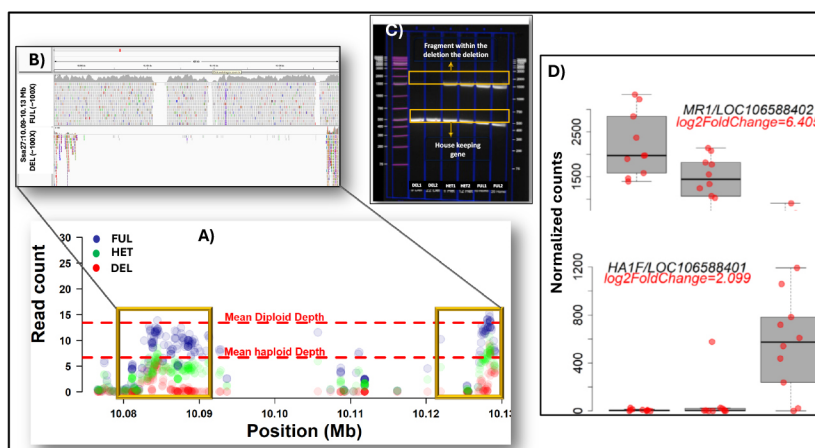


Figure 1: **A)** Copy number variation analysis identified a putative deletion within the QTL region on chromosome 27 in the DEL group. **B)** Integrative Genomics Viewer visualization shows near-complete loss of read coverage across the region in DEL compared to FUL. **C)** PCR validation confirms the deletion, with no amplification in DEL group. **D)** Transcriptome analysis revealed concordant differentially expressed genes with fine-mapping results, supporting the findings.

identified *MRI* and *HA1F* as the only significantly regulated genes between FUL and DEL groups. Specifically, *MRI* was strongly upregulated in susceptible fish, whereas *HA1F* exhibited markedly reduced expression. Proteomic analyses corroborated these findings, demonstrating significantly elevated levels of the *MRI* protein product (*A0A1S3PYN4*) in the DEL group. The concordant evidence from fine-mapping, structural variant detection, transcriptomics, and proteomics strongly supports a central role for *MRI* and *HA1F* in resistance to CMS. These results suggest that dysregulation of key immune pathways, potentially driven by structural variation, may impair host defence mechanisms, with reduced *HA1F* expression likely contributing to compromised immunity in susceptible individuals.

Acknowledgment

The results produced in current study are a part of “CMS-Edit” and the “Genomics4Robust” projects. CMS-Edit project was funded by The Research Council of Norway under grant agreement number 294504 while Genomics4Robust project is an internally funded project from Nofima AS under project number 13281.

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INFLUENCE OF LATITUDE-SPECIFIC LIGHT REGIMES ON LETTUCE PRODUCTION IN DECOUPLED AQUAPONIC SYSTEMS UNDER CONTROLLED ENVIRONMENT AGRICULTURE

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Introduction

Controlled environment agriculture (CEA) enables resource-efficient food production through precise regulation of environmental conditions. Decoupled aquaponic systems (DAPS) provide a flexible production approach by separating fish and plant components, allowing improved control over nutrient delivery and system optimisation. However, how such systems respond to location-specific environmental conditions, particularly light availability, remains insufficiently understood.

Materials and Methods

Lettuce (*Lactuca sativa*) was cultivated in an NFT-based decoupled aquaponic system using water from a tilapia (*Oreochromis niloticus*) recirculating aquaculture system under controlled light environments simulating latitude-dependent conditions of Oslo, Berlin, and Madrid. The experiment was conducted in four consecutive cultivation rounds, each lasting three weeks. Three photosynthetic photon flux density (PPFD) levels (approximately 491, 625, and 815 $\mu\text{mol m}^{-2} \text{s}^{-1}$) were applied under a 12-hour photoperiod using LED lighting. Three cultivation systems were compared: hydroponics (HP), conventional decoupled aquaponics (CAP), and nutrient-balanced decoupled aquaponics (DAP). Plant growth and water-use efficiency was evaluated across treatments.

Results

Plant growth was influenced by both light regime and cultivation system. DAP and HP systems showed comparable biomass production and water-use efficiency, while CAP consistently performed lower across all light conditions. Among the tested scenarios, moderate light conditions (Berlin simulation) resulted in the most balanced plant growth across systems.

Conclusion

The results show that incorporating location-specific light variability is important for optimizing aquaponic system performance. Nutrient-balanced decoupled aquaponic systems can achieve productivity comparable to hydroponics under controlled conditions. These findings support the development of predictive, location-adapted aquaponic designs to improve resource-use efficiency and system stability in controlled environment agriculture.

Acknowledgments

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COMMUNICATING SCIENCE FROM INDUSTRY: BUILDING TRUST, AVOIDING BIAS, AND CREATING GENUINE ENGAGEMENT

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Introduction

In a context where science communication is increasingly scrutinised for conflicts of interest, industry actors are facing a particular challenge: how to share rigorous, evidence-based findings while maintaining credibility and fostering real engagement with the scientific community. This tension is particularly acute in animal nutrition and aquaculture, where commercial stakes are high and the line between scientific communication and marketing can appear blurred. However, industry generates a significant volume of applied research that, if communicated responsibly, can make a genuine contribution to the collective knowledge base. This perspective abstract draws on our experience as an insect ingredient company to reflect on the formats, principles and pitfalls of industry science communication.

A growing toolkit for industry-led science

Industry has progressively developed a range of approaches to generate credible, communicable evidence. Collaborative PhD programmes allow companies to engage with academic institutions on questions that require expertise, equipment, infrastructures or methodological experience beyond internal capacity. These partnerships are mutually beneficial: industry gains access to independent scientific validation, while academic teams engage with real-world research questions and datasets. Research consortia represent a further step, bringing together actors from across the value chain under the scientific oversight of dedicated research institutions. Projects of this kind, where feed manufacturers, producers, ingredient suppliers and scientists work on shared challenges with agreed methodologies, offer a model for generating evidence that is both industrially relevant and scientifically robust. For more targeted questions, partnerships with contract research organisations (CROs) provide controlled, reproducible trial conditions to generate new results and build product-specific claims.

The credibility challenge: trust, bias and transparency

Each collaboration format carries its own credibility profile and its own risks. Funding source, study design, choice of comparators, and the selective reporting of favourable results are all legitimate concerns that the scientific community raises when evaluating industry-generated evidence. These concerns are not unfounded, and industry communicators must engage with them directly rather than defensively. In practice, this means being transparent about methodologies used, trial conditions and their limitations, and clearly distinguishing between findings that are statistically robust and those that are preliminary or observational.

As white papers have emerged as a valuable communication format, enabling companies to share structured, evidence-based content with professional and scientific audiences outside the traditional peer-reviewed publication route, we must also make sure to comply with the above guidelines.

Conferences such as this one play a central role in connecting actors, providing a space where industry and academic scientists can exchange on equal footing.

Submitting work to peer review, even when the timeline is challenging, remains the gold standard. Where that is not possible, applying the same standards of transparency and rigour to other formats is both achievable and necessary.

Reaching beyond the scientific community

Engaging peer scientists is only part of the communication challenge. Industry actors are also well positioned to act as translators of science, bringing evidence-based knowledge to broader audiences including farmers, feed manufacturers, policymakers and the general public. This requires a different set of skills and formats than those used in academic communication, and one that industry, with its commercial communication experience, can genuinely contribute to.

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Social media and short-form video have opened new channels for science communication that the aquaculture and animal nutrition sectors are only beginning to explore. A well-crafted post explaining a key result, or a short video clip from a trial site or processing facility, or even an impactful infographic or simplified visual can reach audiences that a conference presentation or peer-reviewed journal article never will. These formats should come with clarity, visual storytelling and a personal voice, all of which sit more naturally with industry communicators than with many academic researchers. Used responsibly, they can build genuine public engagement.

Conclusion

Industry has a legitimate and important role to play in science communication, provided it is exercised with honesty and methodological discipline. Today's range of formats, from peer-reviewed publications and white papers to social media, creates real opportunities to contribute meaningfully to scientific debate and make results more widely accessible.

The credibility of these contributions depends not on the absence of commercial interest, which is neither realistic nor required, but on the transparency with which that interest is declared and the rigour with which the science is conducted and reported. Building that trustworthy science-backed communication is a long-term investment, but one that benefits the entire field.

Industry is not a passive beneficiary of scientific progress. It is increasingly an active participant in generating, communicating and contextualising it. Embracing this role across all available channels, from academic journals to digital platforms, is both an opportunity and a responsibility.

HISTOPATHOLOGY OF THREE ECONOMICALLY IMPORTANT MARINE FISH SPECIES FROM SELECTED ESTUARIES OF THE GULF OF GUINEA FOR SUSTAINABLE FISHERIES MANAGEMENT

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Introduction

The health of fish populations is closely linked to the quality of their environment, particularly in estuarine ecosystems where natural and human-induced changes can influence aquatic life. Histopathological examination of fish tissues provides valuable information on the effects of environmental stressors and serves as an important tool for monitoring ecosystem health. This study assessed the liver and gonadal tissues of three commercially important marine fish species, *Ilisha africana*, *Cynoglossus senegalensis*, and *Scomberomorus tritor*, collected from the Benin, Brass, and Escravos estuaries of the Gulf of Guinea, Nigeria. Selected water quality parameters were also evaluated to provide baseline information for environmental monitoring and sustainable fisheries management.

Materials and Methods

Fish samples were collected from the three estuaries, and a total of eighteen liver and gonad tissue samples were examined using standard histological procedures. Tissues were fixed in 10% neutral buffered formalin, processed using paraffin embedding techniques, sectioned, and stained with haematoxylin and eosin for microscopic examination. Histopathological alterations were assessed using Histopathological Alteration Index (HAI) and Gonadal Alteration Index (GAI) values. Water quality parameters including temperature, dissolved oxygen, pH, salinity, turbidity, total suspended solids, and nitrate concentration were measured using standard laboratory and field methods.

Results and Discussion

Microscopic examination showed that *Ilisha africana* and *Scomberomorus tritor* maintained normal liver and gonadal structures across all sampled estuaries. Their tissues displayed well-organized cellular arrangements with no visible lesions, resulting in HAI and GAI values of zero.

In contrast, *Cynoglossus senegalensis* showed mild histopathological alterations in both liver and gonad tissues. The observed changes included slight sinusoidal congestion, cytoplasmic vacuolation, and mild germ-cell degeneration. These lesions occurred at a frequency of 33.3%, with mean lesion scores of 1.33 ± 1.15 and HAI and GAI values of 4. Although these findings indicate exposure to low levels of environmental stress, the alterations were minor and unlikely to have significantly affected organ function.

Water quality conditions across the estuaries were generally suitable for fish survival and growth. Temperature ranged from 26.83–27.42 °C, dissolved oxygen from 6.08–6.13 mg L⁻¹, pH from 7.32–7.88, salinity from 21.75–24.92 ppt, turbidity from 12.67–17.50 NTU, total suspended solids from 35.67–46.67 mg L⁻¹, and nitrate concentrations from 1.60–1.85 mg L⁻¹. Most parameters showed no significant spatial variation, although pH differed significantly among locations ($p < 0.05$). Correlation analysis further revealed that salinity played a major role in influencing other environmental variables within the estuarine ecosystem.

Overall, the absence of severe tissue damage suggests that the Benin, Brass, and Escravos estuaries currently provide favourable environmental conditions for fish health. However, the recurring mild alterations observed in *Cynoglossus senegalensis* indicate that this species may be more sensitive to environmental changes and could serve as a useful bioindicator for future monitoring programmes.

Table 1. Summary of Histopathological Findings

Species	Liver Condition	Gonad Condition	HAI	GAI
<i>Ilisha africana</i>	Normal	Normal	0	0
<i>Cynoglossus senegalensis</i>	Mild congestion and vacuolation	Mild germ-cell degeneration	4	4
<i>Scomberomorus tritor</i>	Normal	Normal	0	0

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Conclusion

The study demonstrated that fish populations inhabiting the Benin, Brass, and Escravos estuaries are generally healthy, with normal liver and gonadal structures observed in most species examined. The mild tissue alterations detected in *Cynoglossus senegalensis* suggest the presence of low-level environmental stress, but not to an extent that threatens fish health or reproductive performance. These findings provide important baseline information for environmental assessment, fisheries conservation, and the sustainable management of estuarine resources in the Gulf of Guinea.

Acknowledgment

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BIOLOGICAL CARBON SINKS IN AQUACULTURE: EVALUATING SEQUESTRATION POTENTIAL AND INTEGRATION INTO CARBON MARKETS

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Interests in carbon markets worldwide across the globe have increased significantly due to the growing urgency of climate change been heightened by climate change mitigation, prompting a reassessment of nature-based solutions needed for the reducing tion of greenhouse gases (GHG) emissions. Wetland environments and forests have long been recognized as carbon sinks, but little is known about how aquaculture helps sequester carbon and how it may be included into carbon credit markets. With a focus on shellfish, seaweed and integrated multi-trophic aquaculture (IMTA), this study examines the potential of aquaculture systems to sequester carbon. This study also examines how prepared various aquaculture systems are to take part in compliance and voluntary carbon markets. Through biological processes including carbonate shell formation, photosynthetic CO² uptake and sedimentary carbon burial, aquaculture can function as a blue carbon sink. The study outlined crucial conditions for inclusion in carbon credit schemes, including additionality, permanence and measurability. It also provided insight into new methodologies and technological developments, including Life Cycle Assessment, AI-based monitoring and blockchain for transparent carbon accounting. Notwithstanding these benefits, a number of barriers still stand in the way of progress, including the high costs of verification for small-scale producers, the restrictive regulatory frameworks, and the lack of scientific clarity surrounding carbon measurement. Policymakers' heightened interest in aquaculture and its potential to significantly impact national climate policies are demonstrated by case studies of blue carbon projects and pilot programs.

To realize this potential fully, the study suggests increased investment in research, capacity building, and policy integration. The aquaculture sector can play an important role in the production of climate-smart food, ensuring environmental restoration and sustainable development by bridging gaps between aquaculture and carbon finance.

ALTERNATIVE PEPTIDE SOURCES AS A DIETARY STRATEGY TO ATTENUATE STRESS SIDE-EFFECTS: AN IMMUNOLOGICAL APPROACH IN EUROPEAN SEABASS LARVAE *Dicentrarchus labrax*

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Introduction

Developing stress-reducing strategies has been a priority for aquaculture experts, from engineers to nutritionists. The inclusion of functional ingredients, both as supplements or even in replacement of others, has been thoroughly investigated in the quest for feed formulations that enhance fish welfare and promote health. Protein hydrolyzates from various raw materials renders the most promising bioactive compounds, which incorporation in feeds for fish larval stages has been reported to reduce the impact of stress factors (Aragão et al. 2022). By replacing both fish protein hydrolysate and fish meal with poultry, feather, yeast, wheat and whey peptide fractions, this study aims at exploring the potential of these ingredients to enhance European seabass post-larvae, *Dicentrarchus labrax*, resilience to stress. In particular, it targets the immune response following exposure to an acute stress.

Material and methods

European seabass larvae (73 DAH, initial weight ≈ 0.3 g) were distributed in fifteen 50 L tanks of a recirculating seawater system to which 5 dietary treatments were randomly assigned in triplicates: a commercial diet serving as the control diet (CTRL), and four CTRL-based diets in which fish protein hydrolysate was replaced with poultry (PH), feather (FH), wheat+whey (WWH) hydrolysates and yeast (Y). After acclimatization, seabass larvae were fed the experimental diets for one week, 5 times a day. At the end of the feeding period, the water column was lowered and fish were kept confined for 30 minutes after which the tank was slowly refilled to the top. The feeding period was extended for one more week following the acute stress. Fish were sampled at the end of the feeding period (0h), 48 hours post-stress (48h) and one week post stress (1w), at which points a triangular portion of the peritoneal cavity containing the digestive tract was dissected and snap frozen for the analysis of lysozyme, peroxidase and bactericidal activities and nitric oxide concentration.

Results and discussion

Both nitric oxide concentration and peroxidase activity were higher in fish sampled one week post stress than in fish sampled before the acute stress for all the dietary treatments except WWH. This transversal time effect points to a natural stress-induced response. Moreover, at one week post stress the response was still not stabilized, and was not attenuated by the dietary treatments. Peroxidase activity of fish fed WWH was the exception, as levels before the acute stress were already higher than those of CTRL-fed fish (Fig. 1). An augmented peroxidase activity is usually associated to cell activation and degranulation. However, and since such pre-stress stimulatory potential was not similarly detected in other immune parameters, complementary approaches are required to ascertain the extent of WWH immune-stimulatory role.

Conclusion

The replacement of fish protein fraction with the selected alternative peptide sources did not significantly modulate (attenuated nor intensified) European seabass larvae immune response to an acute stress. Moreover, the majority of the dietary treatments did not affect the immune status prior to stress, with the exception of WWH. This might be regarded as an indication of the tested diets safety regarding a putative inflammatory potential. Additional investigation with more functional endpoints is nevertheless required for a more complete assessment of these alternative protein fractions' stress-counteracting potential.

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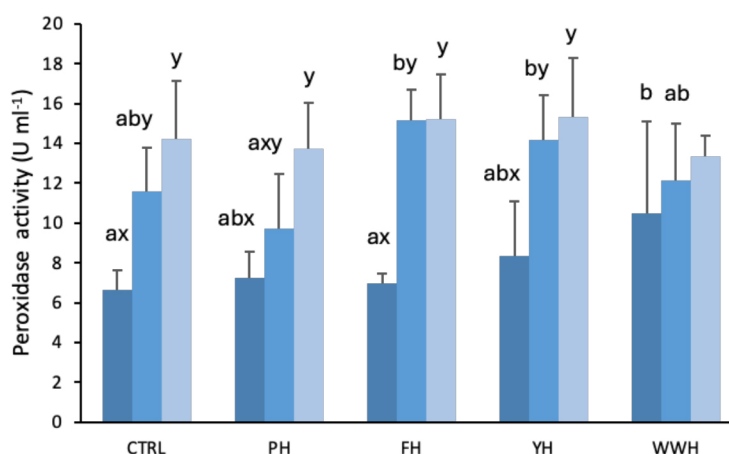


Figure 1. Peroxidase activity in the viscera of European seabass larvae fed experimental diets and sampled at the end of the feeding period (0h ■), 48h following a 30-minutes acute stress (48h ■) and one week post stress (1w ■) (means $\pm$ SD). a and b stand for significant differences attributed to dietary treatment; x,y,z stand for significant differences attributed to sampling time. Two-way ANOVA followed by Tukey post-hoc test ($p \leq 0.05$).

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DIP VACCINATION INDUCES ACUTE STRESS AND DIFFERENTIAL RESPONSES DEPENDING ON THE SPECIES: A COMPARISON AMONG EUROPEAN SEABASS (*Dicentrarchus labrax*), RAINBOW TROUT (*Oncorhynchus mykiss*) AND ATLANTIC SALMON (*Salmo salar*)

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Introduction

Vaccination is a common and necessary practice in aquaculture, since it is one of the most effective tools to prevent infectious disease outbreaks. However, vaccination practices can be fastidious and time consuming, where fish have to be netted and handled by different operators, causing stress to the animals and disrupting normal daily operations. Amongst different vaccination approaches, dip vaccination is minimally invasive, avoids the use of anaesthetics and requires a lesser amount of human intervention and handling than intraperitoneal vaccines. Nonetheless, both the vaccine itself, normally consisting in highly concentrated viscous substances immiscible in water which reduce the efficacy of oxygen capture and exchange, the netting and crowding required for fish immunization, create stressful conditions that can impact fish health. The present work summarizes the main results from three studies aimed to evaluate the physiologic and metabolic effects of acute stress induced by dip vaccination in three juvenile fish species: European seabass (*Dicentrarchus labrax*), rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) pre-smolts.

Material and Methods

Briefly, European seabass (32 g IBW), Atlantic salmon (6-7 g IBW) and rainbow trout (4-5 g IBW) were randomly distributed between tanks and acclimated for one week, all fish were fed commercial diets. Environmental conditions were kept constant, with a temperature of 20°C and salinity of 35 ppt for seabass, whereas salmonid species were maintained at 16°C (rainbow trout) and 14°C (Atlantic salmon) in freshwater. Sampling procedures were transversal to all trials. Before vaccination, samples were collected constituting the time 0 (pre-challenge). Afterwards, fish were netted and transferred into a smaller tank containing the vaccine solution in the case of the vaccine group (VAC) or only water in the case of sham group (SHAM). European seabass were vaccinated with an experimental vaccine containing *Tenacibaculum maritimum* antigens, whereas trout and salmon fingerlings were vaccinated with a commercial one (Aquavac ERM) against *Yersinia ruckeri*. Both groups remained in the tank with aeration for 30 seconds (European seabass) or 1 min (salmonid species). Fish were returned to their initial tanks and samples were collected at 1-, 6-, and 24-hours post-vaccination. At every sampling point, blood, plasma and liver were sampled for the analysis of humoral parameters, metabolites and hepatic oxidative stress response. Brain, hypophysis and head-kidney were collected to assess neuroendocrine and immune responses according to previously defined molecular markers. Skin mucus and water were sampled both for proteomic and transcriptomic (miRNA) analysis, while skin tissue was collected solely for transcriptomics (RNAseq).

Results and discussion

European seabass plasma cortisol levels increased in both vaccinated and sham groups 1-hour after stress and gradually decreased reaching basal levels after 24h. Plasma glucose showed a similar pattern to cortisol, however, glucose levels remained higher in the VAC group compared to SHAM, returning to baseline after 24 hours. Finally, after 1 hour of vaccination plasma lactate levels increased in both groups, but the increase was significantly higher in vaccinated fish. However, this effect disappeared after 6 hours, with lactate levels returning to their baseline. In trout juveniles, following the vaccination procedure, cortisol levels increased in VAC group at 6 hours post-vaccination returning to basal levels after 24h, SHAM group levels remained unchanged. In addition to that, lactate and glucose followed a similar pattern, showing an increase at 6 hours post-vaccination in VAC group. Regarding, those same parameters in SHAM group, glucose increased after 1 hour and 24 hours post-vaccination, while lactate remained unaffected when compared to CTRL group.

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Monocyte counts and plasma nitric oxide (NO) levels in European seabass progressively decreased over time in stressed groups (VAC and SHAM). High glucocorticoid levels can trigger monocyte migration from blood to peripheral tissues. Contrarily, in rainbow trout there is an increase in monocytes 24h post-vaccination in SHAM group, which coincided with a concomitant increase in NO production. Monocytes are NO producing cells and higher or lower NO levels are directly related to cell activation and migration, possibly mediated by cortisol.

Seabass hepatic GSH/GSSG ratio showed a significant drop at 24h in both SHAM and VAC groups, with significantly lower values when compared to earlier time points and to CTRL. The decrease of hepatic GSH/GSSG ratio at 24 h, indicates oxidative stress in the liver. Lower GSH/GSSG ratio values could point to excess ROS production. The decline was higher in vaccinated fish than in SHAM group, suggesting that the immune activation triggered by the antigen increased oxidative demands beyond handling stress alone. On the other hand, trout hepatic GSH/GSSG ratio remained unaffected throughout the experiment for both groups (SHAM and VAC).

These findings indicate an acute stress response immediately after the vaccination procedure in European seabass with a subsequent recovery overtime and a delayed and milder stress response in rainbow trout. The integration of all generated data, is expected to provide better understanding of stress and health related mechanisms involved in vaccination induced stress, namely the relation between the HPI axis and the immune mechanisms. Atlantic salmon samples are currently under evaluation.

Acknowledgments

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PRELIMINARY RESULTS ON REPEATED NONLETHAL HEAT SHOCK ON JUVENILE ASIAN SEABASS *Lates calcarifer*

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Introduction

Juvenile Asian seabass (*Lates calcarifer*) in intensive hatcheries is highly susceptible to bacterial infections, and there is growing interest in non-antibiotic approaches such as non-lethal heat shock (NLHS) to improve fish robustness. This study presents preliminary findings evaluating whether repeated NLHS can enhance survival and modulate stress and immune responses in seabass juveniles exposed to commercial hatchery water containing natural environmental microbiome (**Fig. 1**).

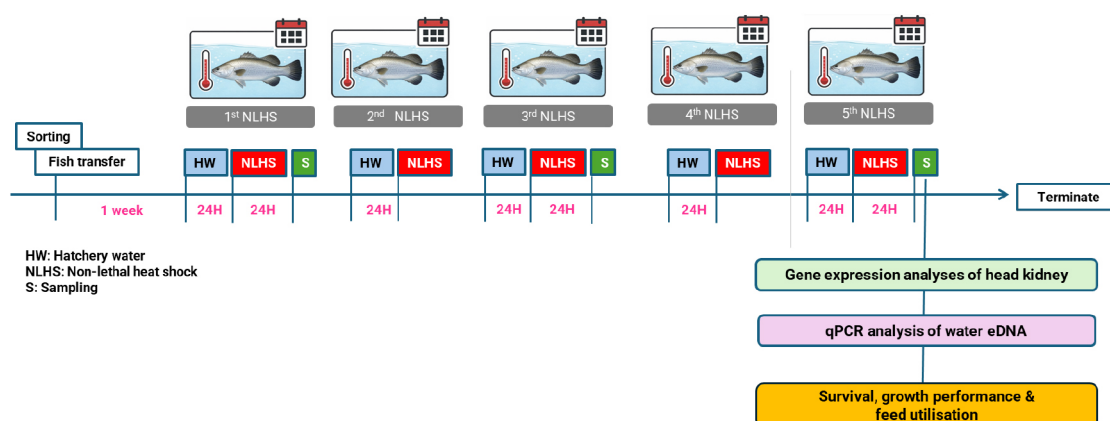
Materials and Methods

Fish (2.90 ± 0.11 g) were reared for 5 weeks in 12 tanks of the BSL2 facility at the National University of Singapore. The experiment followed a 2×2 factorial design, with or without weekly NLHS (10 °C increase for 1 h) and with or without weekly additions of hatchery water as a mock infection. Growth performance and feed utilisation were recorded, and tissue (head kidney) were sampled for quantitative PCR analysis of heat-shock, antioxidant and immune genes. In parallel, tank water, asymptomatic and symptomatic fish were screened by PCR for key bacterial and viral pathogens. NLHS had a significant positive effect on fish survival ($p = 0.002$).

Results and Discussion

NLHS had a significant positive effect on fish survival ($p = 0.002$). In absence of NLHS, the mock infection with hatchery water, had a negative effect on fish survival ($p = 0.864$). NLHS did not significantly alter final body weight, feed conversion ratio or thermal-unit growth coefficient, demonstrating good tolerability of the conditioning protocol. Preliminary head-kidney gene expression results indicate NLHS-associated up-regulation of heat-shock proteins and selected immune markers. Collectively, these findings show that appropriately managed NLHS can improve time-dependent survival of juvenile seabass during realistic hatchery-water exposure, supporting its application as a practical, non-chemical tool for health management in marine finfish hatcheries.

Figure 1



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ECOPHYSIOLOGICAL VULNERABILITY OF THE CLAM *Polititapes rhomboides* TO TURBULENCE AND HEAT STRESS

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Introduction

Infaunal organisms inhabiting marine sediments serve as valuable ecological indicators, offering insights for the influence of key environmental factors in such habitats. Ecosystem health for these habitats subjected to wave storm events with different magnitude in frequency or intensity is reflected in several aspects like species composition, biodiversity, pollution tolerance, or resilience to physical disturbance among others. High-energy environments like wave storms may compromise survival and ecophysiology of inhabitants with potential to disrupt trophic interactions within the benthic food web. Here, we proposed to investigate the impact of high-energy environments on infaunal organisms, monitoring mortality events but also the importance of sublethal effects that may threaten or imbalance metabolism, energetics and surviving bivalve's health. The clam *Polititapes rhomboides* was the target species, creating different physical disturbance environments (short- and long-term scenarios) followed by an additive abiotic stressor (seawater warming, +3°C). Altogether, behavioral, ecophysiological, and stress-related immune responses were monitored, resembling natural drivers impact on historic mortality events of this infaunal population.

Results and Discussion

Mortality events of the clam's populations subjected to sediment mobilization was noted for 16 and 24 days of perturbation (but not for 8 days), representing 14% and 24% of the population, respectively. In all cases, mortality was accelerated with temperature increase reaching 41% for the worst scenario, temperature increase after 24 days of sediment disturbance. In any case, mortality was not linked to rickettsias proliferation or abundance as experimental colony densities remained extremely low and far below the >160 colonies/mm² recorded during natural peak mortality episodes (Darriba et al., 2012; Villaceros-Robineau et al. 2022). As a general pattern, valve opening behaviour declined significantly (15-25%) during sediment mobilization with minor differences among perturbation exposure time (8, 16 and 24 days). Temperature increase at the end of these high-energy exposure periods did not suppose any significant detrimental effect for clam's valve behaviour. Interestingly, primary valve opening behaviour which precedes the rest of the vital functions showed a significant correlation with feeding patterns that have not been observed in other bivalves (Comeau et al. 2018). It is plausible to speculate on such biological correlation for another trophic niche (e.g. infaunal disposition with siphons fully extended along sediment surface).

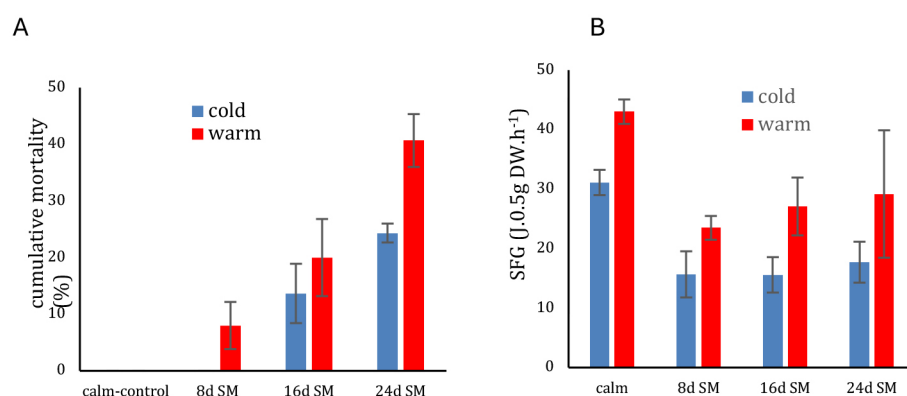


Figure 1. Cumulative mortality (%;A) and Scope For Growth (SFG; B) of the clams subjected to short- and long-term physical disturbance and seawater warming.

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Physical perturbation of individuals caused a significant decrease in feeding and metabolic rates (approximately half of calm scenario) although posterior seawater warming activated also time-specific responses (increase) in both physiological parameters based on optimal food availability set-up selected. Projected energetic status showed a significant degree of vulnerability for disturbed populations based on scope for growth decreased (SFG index; Figure 1B). The cessation of such physical disturbance, together with seawater warming, led to a relatively short-term increase in feeding rates—supported by the availability of optimal food resources—to restore energetic status, although still far from the undisturbed scenario. Combined sediment mobilization and seawater warming significantly increased nitric oxide production after each perturbation cycle (8, 16, or 24 days), with stronger effects following the longest disturbance. Clams under calm conditions did not show nitric oxide increase, even at high temperature, indicating disturbance as the main stressor. A comprehensive scheme of behavioural, physiological and immune-system shifts that infaunal populations experience when subjected to key abiotic stressors responsible of mortality events in the natural environment is discussed. Such knowledge is necessary not only for maintaining biodiversity in our coastlines but also relevant for the socioeconomic sector associated as well as understanding the set of ecosystem services being supported by this and other infaunal species.

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A DIGITAL TWIN FOR IMPLEMENTING THE PRECISION FISH FARMING APPROACH IN RAS

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Introduction

The Precision Fish Farming (PFF) approach integrates sensors, predictive models and decision support systems in four-phase-cycle which include: 1) Observe: real time data from the sensors; 2) Interpret: predictive models to retrieve information from the system; 3) Decide: artificial intelligence (AI) & decision support system; 4) Act: automation & output correction. According to (Fore&al., 2024), the Digital Twin (DT) concept represents a suitable framework for implementing the PFF in cage culture. In this paper, we extend the approach to Recirculation Aquaculture Systems (RAS) and propose a web-based application for allowing end users to test the Knowledge Based Model component of the DT. This study was developed in the framework of the Italy Slovenia INTERREG project “Circular Rainbow”, aimed at promoting the transition from Flow Through System to Hybrid Flow Systems and RAS in trout farming. The latter would lead to enhanced circularity, better control of water quality and hence fish welfare, higher resilience to the effect of climate change on water quantity and quality.

Materials and methods

A DT can be defined as “a virtual representation of a physical asset enabled through data and simulators for visualising, predicting, monitoring, optimising and controlling system states, and improved [decision making](#)”. It includes three main components: i) an observation system; ii) Knowledge Based Models (KBM); iii) data driven models. Taking into account the typical structure and functionality of a RAS, the KBM component included a Mass Budget (MB) and an Energy Budget (EB) module. The data driven component, thus far, has been implemented only for improving the water temperature prediction of the EB module using the Extended Kalman Filter. The MB module includes biotic and abiotic variables, namely “Mean weight of the fish”, “Number of fish”, “Total ammonia nitrogen”, “Nitrate”, “Dissolved oxygen”, “Dissolved inorganic carbon”, “Alkalinity” and “Total suspended solids”. The MB module simulates the evolution of the fish biomass, water circulation, chemical and biochemical processes, deposition of suspended solids. Most processes are affected by water temperature, which is the output of the EB module. The main forcings/controls are: i) feed ration; ii) oxygen supply; iii) air temperature and humidity; iv) quantity and quality of make-up water; v) addition of a base, e.g. NaHCO₃, to control pH. The DT was tested on a pilot RAS located in Pagnacco (Udine, Italy). The observation system included automatic sensors for detecting water temperature, dissolved oxygen, pH, CO₂ and energy use of the most relevant devices, e.g. heat pump, every 15 minutes. This data set was complemented by the weekly sampling of TAN, nitrate, Reactive Phosphorus, Alkalinity. The KBM was coded using R: from the mathematical point of view, the code solved a system of Dynamyc Algebraic Equations including 8 * 16 state variables, where 16 is the number of components of the pilot RAS, as pH and the speciation of DIC and DIN are simulated assuming chemical equilibrium. A demo version of the KBM is freely available at the web interface DiGiTrout: <https://bluefarm.shinyapps.io/DiGiTrout/>, which includes also KBM of FTS and HFS virtual farms. This interface was developed in R (v4.5.1) using the Shiny package (v1.11.1).

Results & discussion

The output of the KBM component of the DT compared well with the data collected in the pilot RAS from October 2025 to February 2026. The growth of rainbow trout was correctly simulated within the weight range 150-500 grams, as shown in Figure 1-a, which compares the model output (line) with the mean weight, averaged over the 12 tanks included in the pilot. The processes affecting the water quality are quite complex: the KBM correctly simulated the trend in nitrate, not shown, related to the increase in fish biomass and, therefore, TAN excretion, thus suggesting the biofilter activity was well reproduced. One of the most relevant model output is pH, which is affected by fish metabolism but depends also on the buffer capacity of the solution: as one can see from Fig. 1b, the trend in pH is correctly simulate both in fish tanks and biofilter: however, the comparison also indicates a slight bias, which should be further investigated. The concentrations of Dissolved oxygen and CO₂ depends also on the efficiency of air blower and degassing units, which were tuned to fit the data.

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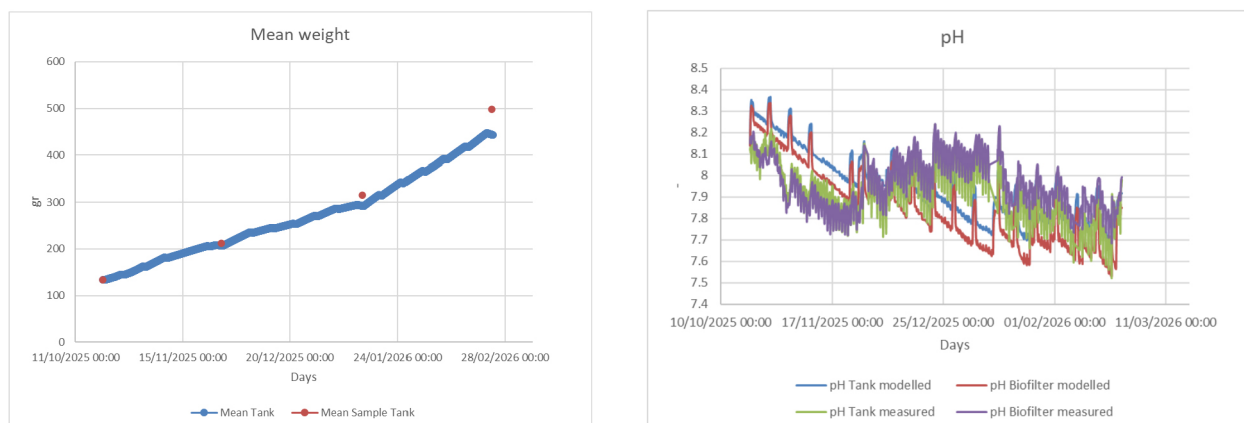


Figure 1: a) Mean weight in all tanks: comparison between model output (line) and manual samples (point).
b) pH in a fish tank and biofilter: comparison between model output (blue and red lines) and data from sensors (violet and green lines)

Concerning the EB module, water temperature and air temperature inside the building were correctly simulated. The Kalman Filter adjusts a parameter used to describe the building transmittance with high precision, with small fluctuations, indicating that the pre-simulation calibration was accurate. Reversing these calculations, the heat provided by the heat pump can be retrieved. This information allows the operator to estimate energy costs related to water cooling/heating.

Overall, the comparison between the KBM component of the DT and the data observed in the Circular Rainbow project indicated that the complex physical, biochemical and chemical processes occurring in a RAS were satisfactorily modelled. On this basis, a prototype of a general KBM for a RAS system was designed, coded and made available through the DiGiTrout web interface, an application which allows one to simulate two RAS management scenarios: 1) daily management (7-day ahead simulation); 2) long term planning (6-month ahead simulation). The web application provides a list of Key Performance Indicators (KPIs), e.g. feed demand, Specific Growth Rate (SGR), fresh water used as input, Feed Conversion Ratio (FCR), total suspended solids collected, dissolved oxygen provided by the air blowers. This information can be used by farmers to improve management. In conclusion, a DT can not only provide a virtual RAS with results comparable to the real systems, but also provide actionable information regarding costs and use of key resources such as feed, fresh water and energy. DT can open new scenarios in the aquaculture sector, optimizing productivity and also allowing farmers to upscale their plant, since forecasts of trends and costs can be generated with high confidence.

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USE OF COLD ATMOSPHERIC PLASMA FOR RAS DECONTAMINATION

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Introduction

Worldwide arising challenges from antibiotic-resistant bacteria require new decontamination strategies. In this regard, the microbial inactivation via reactive oxygen and nitrogen species, provided by the technology of cold atmospheric plasma (CAP) can be applied in recirculating aquaculture systems (RAS) to prevent future disease outbreaks. The decontamination properties of CAP in RAS showed promising results in the past (Balazinski, Hahn et al. 2025). In addition, toxicity tests performed in-vitro on fish cells confirmed a safe application (Balazinski, Hahn et al. 2026). For the consideration of this technology in real environment, an animal testing setup was performed to obtain in-depth results on fish welfare and impact on the water microbiome.

Materials and Methods

The experimental trial was carried out with two equally equipped RAS setups (Figure 1). Each consisted of two tanks with 90 L each, followed by a drum filter, a biofilter and a decontamination unit which was either ultraviolet radiation (UV) or CAP. Each setup had a total capacity of 580 L. In each tank approximately 70 rainbow trout were transferred. The experiment ran for three weeks. For the UV setup two UV lamps each of 55 W were operated continuously. In contrast, CAP was applied daily for 8 h in cycles of 10 min on and 30 min off, corresponding to 2.03 hours of treatment per day. During the experimental trial, water samples were taken at least once a week to determine the microbial load and microbiome in the water, the ionic composition of the water and also the stress of the fish. Standard water parameters, such as oxygen, conductivity, pH and temperature were recorded by a data logger every 15 minutes.

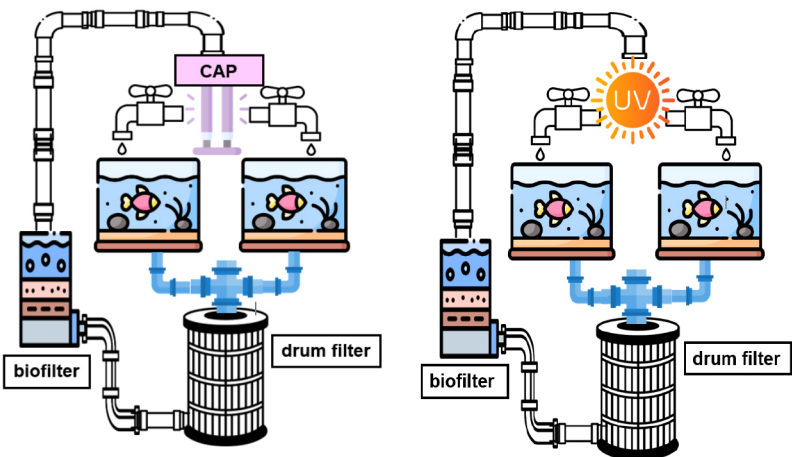


Figure 1: RAS setup with (A) CAP and (B) UV as decontamination unit

Table 1: Comparison of CAP and UV (Average energy consumption [W], Daily device runtime [h], Average microbial load [cfu/mL]), n= 20

	Average energy consumption [W]	Daily device runtime [h]	Average microbial load [cfu/mL]
CAP setup	500	2.03	2.16E+04 (±2.66E+04)
UV setup	110	24.00	3.27E+04 (±5.03E+04)

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Results

The evaluation of the water samples revealed only negligible deviations between the two setups, resulting in an average microbial load of four orders of magnitude (Table 1). For the microbiome analyses the highest abundance in CAP and UV was given by the phylum Pseudomonadota, with a mean abundance of $32\% \pm 15\%$ for CAP and $57\% \pm 2\%$ for UV. Regarding the water composition it is noteworthy, that the pH, conductivity and oxygen values remained stable. Cortisol measurements showed no differences between the two groups.

Discussion

The implementation of CAP in RAS showed, that a sufficient microbial inactivation can be achieved with far shorter treatment periods in contrast to the UV application. In this regards, CAP provides a powerful tool for future RAS decontamination, without affecting major water parameters, such as oxygen, conductivity, pH and temperature. In addition, the composition of the water microbiome was constant, indicating optimal cultivation conditions. However, the experiments were successfully performed with rainbow trout, further testing must be carried out to obtain optimal settings for other species and setups.

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DIETARY SUPPLEMENTATION WITH UNPROCESSED LIQUID *Spirulina platensis* CULTURE ENHANCES GROWTH, FLESH QUALITY, AND ANTIOXIDANT CAPACITY IN COMMON CARP (*Cyprinus carpio*)

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Introduction

Microalgae have attracted growing attention as functional feed ingredients in aquaculture, as a result of their favourable nutritional composition and richness in bioactive compounds. *Spirulina platensis* is well known for its high protein content, essential amino acid profile, and antioxidant pigments such as phycocyanin and carotenoids (Bortolini *et al.*, 2022). The chemical composition of *Spirulina platensis* includes approximately 53% crude protein, 6.6% crude lipids, and 15.3% soluble carbohydrates, along with significant levels of B-complex vitamins, vitamin E, and minerals such as potassium and iron (Michael *et al.*, 2019; Alagawany *et al.*, 2021). Previous studies have reported beneficial effects of powdered *Spirulina platensis* at 3–10% dietary inclusion on growth and health of various fish species, including salmon (Roohani *et al.*, 2019), koi carp (Napolitano *et al.*, 2022), and common carp (Ahmed, 2023), but these have exclusively used dried biomass. Most studies to date have relied on dried or powdered spirulina biomass, which requires energy-intensive harvesting, centrifugation, and drying steps. Using the liquid culture directly, algal biomass together with its growth medium, could substantially reduce processing costs while preserving heat-sensitive bioactive molecules. Cultivating *S. platensis* on recirculating aquaculture system (RAS) wastewater integrates nutrient recycling into the production chain. As an important freshwater aquaculture species in Romania, common carp (*Cyprinus carpio*) provides a suitable model for evaluating such novel feed strategies. This study investigated if dietary inclusion of unprocessed liquid *S. platensis* culture at 5%, 10%, and 15% can register comparable growth, health, and antioxidant benefits to those reported for conventional spirulina powder.

Materials and Methods

Spirulina platensis was cultivated in 1000 L tanks filled with 90% RAS wastewater and 10% Zarouk medium, illuminated with a 300 W LED lamp, and harvested at the stationary phase two weeks after inoculation (biomass: 1.04 ± 0.02 g L⁻¹ absolute dry weight). Juvenile carp (initial weight 11.01 ± 0.9 g) were randomly assigned to 12 tanks in a RAS facility (30 fish per tank, three replicates per treatment) and fed one of four isonitrogenous diets for 12 weeks: a control diet (15% water top-dressing), V1 (10% water + 5% liquid spirulina), V2 (5% water + 10% liquid spirulina), or V3 (15% liquid spirulina). Fish were fed at 3% body weight day⁻¹ in three daily meals. At the end of the trial, growth performance, feed utilisation, somatic indices, flesh proximate composition (NIR analysis), haematological profile (automated haematology analyser), and tissue-level antioxidant enzyme activities (SOD, CAT, GPx) and lipid peroxidation (MDA) were assessed.

Results and Discussion

All spirulina-supplemented groups showed significantly higher weight gain than the control in weight gain ($p \leq 0.001$), feed conversion ratio, specific growth rate, and relative growth rate, with V2 (10% inclusion) achieving the best overall performance. Polynomial contrast analysis confirmed an initially near-linear improvement followed by a plateau at 15% inclusion, meaning that 15% did not improve results beyond 10%. CF, HSI, and VSI did not differ significantly between groups, which eliminates negative effects on fish condition. Flesh protein content increased progressively from 13.68% (control) to 14.72% (V3), while lipid content declined from 14.48% to 10.22%, which means leaner fillets with higher protein value. Collagen content also rose with increasing spirulina levels. Hematological responses were dose-dependent: white blood cell counts peaked in V1 and decreased at 15% inclusion, while hemoglobin concentrations were higher in supplemented groups, consistent with the known erythropoietic effects of spirulina's iron and phycocyanin content (Ahmed, 2023). Antioxidant enzyme activities increased across muscle, liver, and intestinal tissues in a dose-dependent manner, with significant elevations in SOD, CAT, and GPx in V2 and V3 groups. MDA levels decreased progressively, indicating reduced oxidative damage. The observed reduction in MDA is consistent with findings in cyprinids supplemented with

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micro- and macroalgae (Abdel-Tawwab *et al.*, 2021; Ahmadifar *et al.*, 2023). Al-Deriny *et al.* (2020) attributed the SOD upregulation in *Nile tilapia* to the polyphenol and carotenoid content of *Spirulina platensis*, which is likely to explain the tissue-level responses observed in the present study. The parallel improvement in growth and antioxidant status suggests that the upregulation of enzymatic defenses represents a beneficial adaptive response rather than a marker of oxidative stress.

Conclusion

Unprocessed liquid *S. platensis* culture grown on RAS wastewater proved to be an effective functional feed ingredient for common carp, delivering growth and health benefits comparable to those reported for dried spirulina. The 10% dietary inclusion registered the optimal balance between growth enhancement, improved flesh composition, and strengthened antioxidant defence. This finding supports the potential of the low-cost, circular approach for practical carp aquaculture.

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CIRCULAR BIOECONOMY IN AQUACULTURE: GROWTH PERFORMANCE, MEAT QUALITY AND HEMATOLOGICAL RESPONSES IN *Cyprinus carpio* FED WITH LIQUID *Chlorella vulgaris*, CULTIVATED ON WASTEWATER FROM A RECIRCULATING SYSTEM

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Circular Bioeconomy and Ecological Rationale

Recirculating aquaculture systems (RAS) generate nutrient-rich wastewater containing elevated concentrations of nitrogen, phosphorus and organic matter, compounds that represent both an environmental burden and an underutilized biological resource. Microalgae, particularly *Chlorella vulgaris*, are efficient biological filters capable of assimilating these compounds while simultaneously producing protein-dense, bioactive biomass. When algae are cultivated on RAS effluent, two objectives are achieved within a single closed loop: the wastewater is biologically depolluted and the resulting biomass is valorized as a functional fish feed ingredient, exemplifying circular bioeconomy principles at the farm scale.

The liquid culture of *C. vulgaris* (LCV) used in this experiment was produced in a 1000 L photobioreactor supplied with 90% RAS wastewater and 10% Tamia-type nutrient medium. Chemical analysis of the RAS effluent confirmed substantial concentrations of ammonium (NH_4^+), nitrite (NO_2^-), phosphate (PO_4^{3-}), calcium (Ca^{2+}) and magnesium (Mg^{2+}), alongside microelements including copper (Cu), nickel (Ni) and manganese (Mn). Following two weeks of cultivation under a 300 W Lumatek LED lamp, the algal culture reached the stationary growth phase (dry biomass $1.05 \pm 0.03 \text{ g L}^{-1}$; crude protein 48%, lipids 14.5%, carbohydrates 19.2%; lysine-rich essential amino acid profile), with concomitant depletion of nutrient loads in the culture medium, confirming the depollution function of the algal culture. By delivering the biomass directly as a liquid suspension sprayed onto commercial pellets, the approach eliminates the energy-intensive steps of centrifugation, drying and storage, further reducing the ecological and economic footprint of the process.

Four isonitrogenous and isolipidic diets were formulated: Control (15% water) and three LCV diets at 5% (V1), 10% (V2) and 15% (V3) inclusion. Juvenile *C. carpio* ($11.39 \pm 0.84 \text{ g}$; $n = 360$; 30 tank⁻¹, triplicate) were reared for 12 weeks in a RAS comprising 12 fiberglass tanks (0.5 m³), fed three times daily at 3% body weight day⁻¹. Growth indices, flesh proximate composition (NIR; DA 7250) and hematological parameters (Abacus Vet 5) were assessed and analyzed by one-way ANOVA with Tukey post-hoc test (SPSS v.21; $p < 0.05$).

Results and Discussion

Growth performance improved in a positive linear fashion with increasing LCV inclusion. Weight gain (WG) and relative growth rate (RGR) were significantly higher in V2 and V3 than Control ($p \leq 0.01$), specific growth rate (SGR) was significantly elevated in V2 ($p \leq 0.05$), and feed conversion ratio (FCR) declined from 3.00 ± 0.05 (Control) to $2.52 \pm 0.06 \text{ g g}^{-1}$ (V3). Somatic indices (condition factor, hepatosomatic index, viscerosomatic index) were unaffected, confirming physiological safety across all inclusion levels.

Flesh quality responded positively: protein content increased from 67.66% (Control) to 70.60% (V2; $p < 0.001$), moisture decreased markedly ($p < 0.001$), and ash content rose with inclusion level ($p < 0.001$), reflecting enhanced mineral deposition consistent with the mineral-rich biochemical profile of *C. vulgaris* biomass produced on RAS wastewater.

Haematological assessment revealed elevated white blood cell (WBC) counts across all LCV groups ($p = 0.001$), haemoglobin concentrations increasing from 115 to 138–140 g L⁻¹ ($p = 0.001$), and dose-dependent increases in erythrocytes and thrombocytes (V3: $0.74 \times 10^{12} \text{ L}^{-1}$ and $27 \times 10^9 \text{ L}^{-1}$, respectively), collectively indicative of immunostimulatory activity consistent with β -glucan and polysaccharide-mediated macrophage activation.

Conclusion

Cultivating *C. vulgaris* on RAS wastewater simultaneously depollutes aquaculture effluents and generates a functional, cost-effective feed ingredient that improves growth, flesh quality and immunohematological status of common carp. By closing the nutrient loop between fish waste and fish feed, the approach offers a scalable, ecologically grounded model for sustainable RAS-based aquaculture aligned with circular bioeconomy principles. Future work should assess metal accumulation dynamics in algal biomass across successive cultivation cycles and evaluate the long-term productive and ecological outcomes at semi-industrial scale.

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HEMATOLOGICAL AND HISTOLOGICAL PROFILE OF JUVENILE *Colossoma macropomum* TREATED WITH PRAZIQUANTEL-ENCAPSULATED CHITOSAN AND MODIFIED ALGINATE NANOPARTICLES FOR MONOGENEAN CONTROL

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Introduction

Aquaculture has been seeking more effective and safer alternatives for parasite control in fish. In this context, the present study evaluates the use of chitosan and modified alginate nanoparticles containing praziquantel for oral administration in juvenile *Colossoma macropomum*, aiming to control monogenean infections and assess potential hematological and histological changes associated with the treatment.

Material and Methods

Chitosan and modified alginate nanoparticles loaded with praziquantel were produced by complex coacervation and administered orally to juvenile *Colossoma macropomum*. Hematological analyses were performed using blood samples collected at 7, 14, and 21 days after treatment, with a control group for comparison. Histological analyses of the intestine and gills were conducted using hematoxylin and eosin (H&E) and periodic acid–Schiff (PAS) staining to evaluate tissue morphology.

Table 1. Hematological profile of *Colossoma macropomum* during the experimental period at 7, 14, and 21 days after treatment. Values are expressed as mean $\pm$ standard deviation (SD).

Parâmetro	7 dias	14 dias	21 dias
Eritrócitos ($10^6/\mu\text{L}$)	$0,14 \pm 0,11$	$0,19 \pm 0,17$	$0,19 \pm 0,17$
Leucócitos ($10^3/\mu\text{L}$)	$137,30 \pm 112,48$	$133,35 \pm 130,26$	$133,35 \pm 130,26$
Hemoglobina (g/dL)	$19,94 \pm 4,73$	$18,80 \pm 3,70$	$18,80 \pm 3,70$
Hematócrito (%)	$59,83 \pm 14,20$	$56,39 \pm 11,11$	$56,39 \pm 11,11$
Linfócitos (%)	$92,55 \pm 2,19$	$91,89 \pm 1,65$	$91,89 \pm 1,65$
Neutrófilos (%)	$3,18 \pm 1,45$	$1,93 \pm 1,30$	$1,93 \pm 1,30$
Monócitos (%)	$4,28 \pm 1,64$	$6,12 \pm 0,96$	$6,12 \pm 0,96$

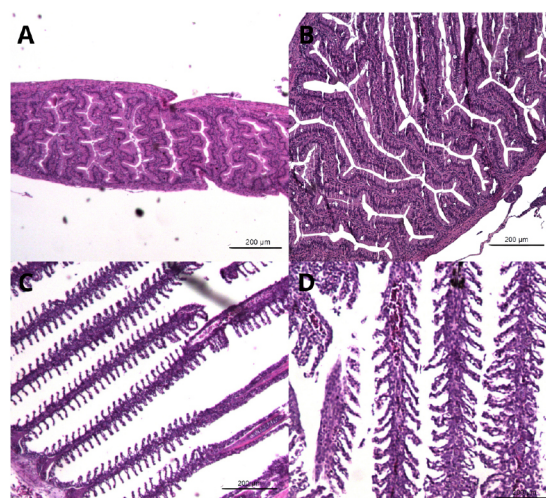


Figure 1. Representative histological sections of the intestine and gills of *Colossoma macropomum* after treatment. (A) Intestine, 7 days; (B) Gills, 7 days; (C) Intestine, 21 days; (D) Gills, 21 days. Hematoxylin and eosin (H&E) staining. Scale bar = 200 μm .

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Discussion and Results

Hematological and histological analyses were performed to evaluate the physiological response and tissue morphology of fish throughout the experimental period, following the methodologies described by Ranzani-Paiva et al. (2013). Hematological analysis revealed slight variations in blood parameters over the sampling period (Table 1). Erythrocyte counts averaged $0.14 \pm 0.11 \times 10^6/\mu\text{L}$, $0.19 \pm 0.17 \times 10^6/\mu\text{L}$, and $0.18 \pm 0.14 \times 10^6/\mu\text{L}$ at 7, 14, and 21 days, respectively. Hematocrit and estimated hemoglobin showed a gradual decrease throughout the experimental period, whereas total leukocyte counts remained similar at 7 and 14 days, with an increase observed at 21 days. Differential leukocyte counts were characterized by a predominance of lymphocytes during all sampling periods, consistent with the hematological evaluation methods proposed for teleost fish by Ranzani-Paiva et al. (2013).

Histological evaluation of the intestine and gills performed at 7 and 21 days after treatment showed preservation of the overall tissue architecture (Figure 1). Intestinal folds and villi remained organized, while the gills maintained the structural organization of the filaments and secondary lamellae, with no marked morphological alterations observed between the evaluated periods. Similar histological approaches have been employed to assess tissue responses in *Colossoma macropomum* exposed to different experimental conditions (Vieira et al., 2019). Overall, the results suggest that the hematological variations observed throughout the experiment were not accompanied by evident histological alterations under the experimental conditions evaluated.

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SUPPORTING METABOLIC HEALTH AND PERFORMANCE IN FISH THROUGH BILE SALT SUPPLEMENTATION

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Introduction

Fish synthesize primary bile acids (BAs) from cholesterol via classical (CYP7A1) and alternative (CYP27A1) pathways, after which they are conjugated and stored in the gallbladder as bile salts (BS). Upon feeding, BS are released to emulsify dietary lipids, enabling efficient hydrolysis and absorption. Most BA are recycled through enterohepatic circulation; although a physiologically relevant fraction is lost in faeces and must be resynthesized (Zhou *et al.*, 2024). In fish, the magnitude of this loss varies with diet and species. Additionally, intensive aquaculture conditions—including handling, high stocking densities, variable water quality, and plantproteinrich feeds—can impair hepatic BA synthesis, gallbladder emptying and recycling efficiency. Soy-derived ingredients may further reduce digestive capacity by diluting taurine and introducing antinutritional factors (Staessen *et al.*, 2021). A diminished BS pool compromises lipid digestion, nutrient utilization, energy balance and growth. This study evaluated whether supplementing diets with bovinederived BS (200 mg/kg) could support BA homeostasis, lipid digestion and production performance in red seabream (*Pagrus major*) and Nile tilapia (*Oreochromis niloticus*) reared under commercial conditions.

Material and Methods

Two independent feeding trials were performed. In Trial 1, juvenile red seabream (initial body weight 151 g) were stocked at 12.8 kg/m³ (12 fish per 140L tank) in a recirculating aquaculture system (RAS) operated at 24 °C. Four replicate tanks per treatment received either a control feed (42% crude protein, 13% fat) or the same feed supplemented with 200 mg/kg bile salts (BS; Lipogest, Adisseo) for 18 weeks. The basal diet included 26% fishmeal, 25% soy protein concentrate, and 10% soybean meal, reflecting a moderate plantprotein inclusion level consistent with commercial formulations. Plasma bile acid (BA) and cholesterol concentrations, growth performance, and feed conversion ratio (FCR) were measured. Because only two treatments were compared, independent twosample ttests were used to evaluate differences ($p < 0.05$).

In Trial 2, Nile tilapia (initial weight 44.8 g) were stocked into ten hapas (2 m × 1 m × 1 m) placed in a reservoir, with five hapas per treatment. Each hapa held 70 fish (1.6 kg/m³). The basal diet contained 28% crude protein and 4% fat, with 33.8% soybean meal and 11.2% poultry meal as the primary proteins and no fishmeal. Fish were fed three meals daily for 10 weeks at 31–35 °C and 5 ppt salinity, reaching ~500 g at harvest. Growth performance, FCR and fillet yield were assessed. Due to potential variance heterogeneity in field conditions, Welch's ttest was applied ($p < 0.05$).

Results

In red seabream, BS supplementation significantly increased plasma BA concentrations by 17% and plasma cholesterol by 44% relative to the control. Elevated plasma cholesterol indicates improved nutrient utilization and a more favourable energy balance. Increased circulating cholesterol also enhances hepatic BA synthesis, expanding the gallbladder BS reservoir. These metabolic improvements translated into superior performance, with BSsupplemented fish exhibiting a significant 7% increase in specific growth rate (SGR) and a numerical 2% decrease in FCR compared to control. In tilapia, BS supplementation resulted in a numerical 2.4% increase in fillet yield and a 4.5% reduction in FCR at harvest. These benefits occurred despite fluctuating temperatures and moderate salinity in the reservoir, demonstrating improved nutrient retention efficiency and physiological robustness.

Discussion

Across species and production systems, dietary supplementation with 200 mg/kg bovine BS (Lipogest, Adisseo) effectively supported BA homeostasis, digestive function and performance. In seabream fed a soyrich diet, increased plasma cholesterol and BA levels indicate enhanced enterohepatic circulation, better gallbladder filling and improved bile availability for lipid emulsification. This resulted in higher feed efficiency and growth. In tilapia reared under warm and moderatesalinity conditions, BS supplementation improved nutrient utilization and fillet yield, suggesting stronger metabolic resilience. The lack of fishmeal and high soybean meal inclusion in the tilapia diet underscores the value of BS supplementation in plantproteinbased formulations, where endogenous BA synthesis and taurine status may be limiting. As aquaculture continues to adopt sustainable, lowfishmeal feeds, BS supplementation represents an effective nutritional tool to maintain bile acid pool adequacy, support hepatic health and optimize production outcomes.

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ENHANCING THE COST EFFICIENCY OF EUROPEAN SEABASS (*Dicentrarchus labrax*) FEEDS THROUGH MARINE PEPTIDE-BASED FORMULATION STRATEGIES

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Introduction

The expansion of aquaculture production is driving the development of feed formulation strategies that improve sustainability, increase resilience to fluctuations of raw material prices, and enhance economic efficiency. Fish meal (FM) remains a key ingredient in marine fish diets due to its nutritional value and palatability; however, sustainability targets and formulation economics are encouraging a progressive reduction in FM inclusion. Consequently, cost-saving strategies increasingly focus on reducing FM levels or utilising alternative FM qualities, provided that growth performance and feed efficiency are maintained. Marine peptide concentrates (MPCs), characterised by high digestibility and functional bioactive properties, offer effective support for these approaches by enhancing nutrient utilisation and growth efficiency. This study evaluated the efficacy of a marine peptide concentrate (Pepsea F) as a functional additive in European seabass (*Dicentrarchus labrax*), assessing its ability to support FM inclusion reduction and FM quality substitution strategies while enhancing biological and economic performance.

Material and Methods

An 11-week feeding trial was conducted using European seabass with an initial mean body weight of 18 g. Fish were reared in a recirculating aquaculture system (RAS) using 150-L tanks stocked with 25 fish per tank under controlled conditions (water temperature 22.7 ± 1.2 °C). Feed was supplied twenty times per day using automatic feeders. Experimental feeds included the marine peptide concentrate, Pepsea F (liquid, 40% crude protein (CP40); Adisseo), at inclusion levels of 0.75%, 1.5% or 2.25% under two cost-reduction strategies: (i) partial replacement of high-quality FM (CP68), reducing inclusion from 20% to 10%, and (ii) substitution of FM quality from CP68 to CP60 at a constant 20% inclusion level. Each treatment was tested in triplicate. All experimental feeds were formulated to be isoproteic, isolipidic and isoenergetic. Growth performance parameters, including feed intake, specific growth rate (SGR), and feed conversion ratio (FCR), were measured. Data were analysed using one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$). An economic assessment based on feed cost, feed efficiency, biomass output, and return on investment (ROI) was conducted using Turkish raw material prices as reference (September 2025).

Results

Reduction of FM inclusion from 20% to 10% (CP68) negatively affected SGR and FCR when no functional additive was applied. However, supplementation with Pepsea F significantly restored and improved performance. Inclusion of 1.5% Pepsea F in the 10% FM diet resulted in a numerical 4% increase in feed intake, 5% in SGR and a 4% improvement in FCR compared to the 20% FM control diet, while simultaneously reducing feed formulation cost by 32 €/t. Improved feed efficiency corresponded to approximately 40 kg of feed saved per metric ton of feed, equivalent to 60 €/t in on-farm savings. Combined with feed formulation savings, total economic benefit reached 92 €/t of feed. In addition, increased growth performance resulted in a 5% increase in final biomass, corresponding to an additional 50 kg of fish per metric ton, equivalent to 325 € of extra income (farm-gate price: 6.5 €/kg). This scenario yielded an ROI of the additive of 8.

In the second strategy, replacement of FM quality from CP68 to CP60 negatively impacted growth and feed efficiency. Supplementation with 0.75% Pepsea F not only mitigated these effects but exceeded the performance of the 20% CP68 control diet, numerically improving feed intake by 1%, SGR by 4% and FCR by 2%. Feed reformulation reduced feed cost by 19 €/t, while improved FCR generated an additional 30 €/t in feed savings for producers (49 €/t combined). Biomass gains generated approximately 260 € of additional income per metric ton of fish produced. This scenario resulted in an ROI of 12.

Discussion

The results demonstrate that Pepsea F effectively compensates for both reduced FM inclusion and downgraded FM quality in European seabass feeds, maintaining or improving growth performance and feed efficiency relative to high-FM control diets. The observed improvements in SGR and FCR highlight the functional role of marine peptides beyond their nutritional contribution, linked to enhanced palatability and nutrient utilisation. Importantly, the substantial ROI values obtained across both formulation strategies confirm the economic relevance of Pepsea F inclusion, delivering benefits at both feed mill and farm levels. Overall, these findings support the use of marine peptide concentrates as sustainable, functional tools to optimise seabass feed formulations, reduce dependence on high-quality fish meal, and enhance profitability in marine aquaculture.

DIETARY LYSOPHOSPHOLIPIDS ENHANCE GROWTH PERFORMANCE AND ASTAXANTHIN DEPOSITION IN ATLANTIC SALMON (*Salmo salar*) AND RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Astaxanthin is an essential carotenoid in salmonid nutrition and a major determinant of flesh pigmentation, directly influencing consumer acceptance and market value. As salmonids are unable to synthesize astaxanthin *de novo*, dietary supplementation is mandatory. Beyond pigmentation, astaxanthin contributes to antioxidant protection, immune modulation, and stress resistance. However, its bioavailability is often limited by its lipophilic nature, low solubility in the intestinal environment, and reliance on passive absorption mechanisms. Nutritional strategies that enhance lipid digestion, emulsification, micelle formation, and postabsorptive transport may therefore improve astaxanthin utilization and retention. Lysophospholipids exhibit superior emulsifying properties compared to conventional phospholipids and are involved in lipoprotein metabolism. This study evaluated the effects of a lysophospholipid-based additive (Aqualyso, Adisseo) on growth performance and fillet pigmentation in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*).

Material and Methods

Two feeding trials were conducted. In the first trial, Atlantic salmon post-smolts (initial body weight 160 g) were reared for 12 weeks in 1.2 m³ tanks (35 fish per tank; three replicates per treatment) under controlled salinity (33 ppt) and temperature (12 ± 2 °C). A basal diet (45% crude protein, 28% lipid) containing 60 ppm astaxanthin and no lecithin was supplemented with graded inclusion levels of liquid Aqualyso (0, 0.05, 0.1, and 0.2%). Fish were fed to apparent satiation. Growth performance parameters were assessed at weeks 6 and 12, and fillet samples were analysed for astaxanthin and total carotenoid concentration to determine pigment retention efficiency.

In the second trial, rainbow trout sub-adults (initial body weight 300 g) were fed for 10 weeks in a flow-through system (1,600 L tanks; 18 fish per tank; two replicates per treatment) at natural water temperatures (13–14 °C). A basal diet (44% crude protein, 25% lipid; 60 ppm astaxanthin) was compared with the same diet supplemented with 0.1% powdered Aqualyso. Growth performance was monitored, and fillet pigmentation was evaluated at weeks 5 and 10 using colourimetric analysis (CIE Lab* system; n=12 fillets per treatment at each sampling point). Statistical analyses were performed using one-way ANOVA or Students T-test, with significance set at $p < 0.05$.

Results

In Atlantic salmon, Aqualyso supplementation improved growth performance in a dose-responsive manner. The 0.1% inclusion level produced the strongest growth response, with numerically higher feed intake (+10%) and specific growth rate (+6%) compared to the control, observable from week 6 onwards, while feed conversion ratio was unaffected. All supplementation levels increased fillet astaxanthin concentration. The greatest improvement in pigment utilization was observed at 0.05% inclusion, resulting in a numerical increase of 32% in fillet astaxanthin concentration and a 29% increase in astaxanthin retention efficiency. Total carotenoid concentration and retention were numerically increased by 24% and 23%, respectively.

In rainbow trout, dietary inclusion of 0.1% Aqualyso numerically increased final body weight by 5% and improved feed conversion ratio by 3%. Colourimetric analysis indicated higher redness (a*) and yellowness (b*) values at week 5, and a significant improvement in hue angle (h) at week 10, indicating enhanced pigmentation efficiency compared to the control.

Discussion

Lysophospholipid supplementation improved growth performance and astaxanthin utilization in both salmonid species. These effects are likely linked to enhanced emulsification, more efficient micelle formation, improved lipid digestion, and increased intestinal absorption of lipophilic compounds such as astaxanthin. This pigmentation benefits were consistently demonstrated through complementary evaluation approaches: biochemical quantification of astaxanthin and carotenoid retention in Atlantic salmon, and objective colourimetric measurements in rainbow trout. Overall, the results support the use of lysophospholipids as an effective nutritional strategy to improve growth and optimize pigment efficiency in salmonid aquaculture.

TRANSCRIPTOMIC INSIGHTS INTO THE ONSET AND MATURATION OF THE CIRCADIAN CLOCK IN EURASIAN PERCH

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Introduction

The circadian clock coordinates multiple aspects of physiology, including digestion (Pal and Maitra, 2018), cell proliferation (Tamai et al., 2012), stress responses (Sánchez-Vázquez et al., 2019), spawning, and behavior (Marchesan et al., 2005). While the molecular architecture of the circadian system is well characterized in adult vertebrates, its developmental onset and early functional organization remain poorly understood, particularly in fish. In teleosts, circadian rhythms have been described at both behavioral and molecular levels (Cuesta et al., 2014); however, studies addressing their ontogeny are still limited. As a result, the timing of initial rhythmicity and the processes underlying the establishment of a functional circadian system during early development remain largely unresolved.

The aim of this study was to determine the onset and progression of circadian rhythmicity in Eurasian perch by combining high-resolution temporal sampling of core clock gene expression with transcriptomic profiling to identify rhythmically expressed genes and associated molecular pathways across key developmental stages.

Results and discussion

Using high-resolution temporal sampling, we demonstrated that core circadian clock genes in Eurasian perch begin to oscillate at 3 days post-hatching (DPH) and exhibit robust rhythmicity by 28 DPH under a controlled photoperiod (14L:10D). These two developmental stages were therefore selected for transcriptomic profiling using 24-hour round-the-clock sampling at 4-hour intervals to capture diel gene expression dynamics and identify molecular pathways involved in the emergence and consolidation of circadian regulation. Using MetaCycle, we identified 1,466 rhythmically expressed genes at 3 DPH and 478 at 28 DPH, of which 206 were shared between both stages, indicating a combination of conserved and stage-specific rhythmic processes.

Temporal clustering with TCseq revealed seven distinct expression clusters at each developmental stage, reflecting diverse rhythmic patterns across the diel cycle. Functional annotation and gene ontology analyses further indicated that these clusters were associated with a range of biological processes, suggesting a progressive organization of circadian-regulated pathways during development. This approach uncovered both shared and stage-specific rhythmic transcripts, including several genes not previously described as circadian biomarkers in fish. Among these were, for example, *ciart* and *cipc*, which are known components of the negative regulatory arm of the circadian clock in other vertebrates but have not yet been characterized in fish.

To further investigate candidate genes associated with early circadian development, we implemented two complementary validation strategies. First, a cross-species approach targeted genes previously reported in mammals or other model organisms but not in fish, including *ciart* and *cipc*, to assess their evolutionary conservation and potential functional relevance in teleost circadian systems. Second, we selected a set of ten putative novel circadian rhythm-associated genes identified from our transcriptomic dataset, representing candidates with no prior links to circadian regulation. Collectively, these findings expand current knowledge of the genetic architecture underlying early circadian development and lay the groundwork for further mechanistic exploration.

(Continued on next page)

Acknowledgment

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TOWARDS STANDARDISED FISH WELFARE ASSESSMENT: DEVELOPMENT OF A DIGITAL MULTI-INDICATOR PLATFORM

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Introduction

Fish welfare assessment is increasingly recognized as a key component of modern aquaculture, but its routine implementation remains challenging. Frameworks such as the Salmon Welfare Index Model and FISHWELL have shown the value of formal assessment using operational welfare indicators (Stien et al., 2013; Noble et al., 2018). However, welfare information is often fragmented across production records, health monitoring and isolated observations. Production key performance indicators are relevant to welfare, but they do not fully capture behavioural expression, environmental suitability or management practices (Martins et al., 2012; Saraiva et al., 2019). This work describes the development and preliminary applicability testing of WAAVE - Welfare Assessment of Aquatic Vertebrates, as a digital multi-indicator workflow for structured fish welfare assessment across aquaculture, research laboratories and public aquaria.

Methodological development

WAAVE was developed by FishEthoGroup as a mobile and web-based workflow grounded in scientific literature and applied welfare research. The indicator catalog integrates animal-based, environmental-based and management-based indicators, including external appearance, feeding behaviour, swimming patterns, respiratory rate, aggression, water quality, light conditions, disturbance, staff training, handling protocols, records and traceability. The workflow was designed to assign indicators according to the selected production or rearing context, allowing aquaculture, laboratory and public aquarium assessments to use a consistent structure while retaining setting- and species-specific relevance.

Indicators are evaluated through a traffic-light scoring system and are manually scored by trained users. The workflow then summarizes the assessment into structured outputs, including an overall welfare score, priority concerns and a report intended to support interpretation and follow-up actions (Figure 1). The aim is not to automate welfare judgement, but to standardize indicator selection, scoring and reporting in a transparent format that can be reviewed and improved through validation.

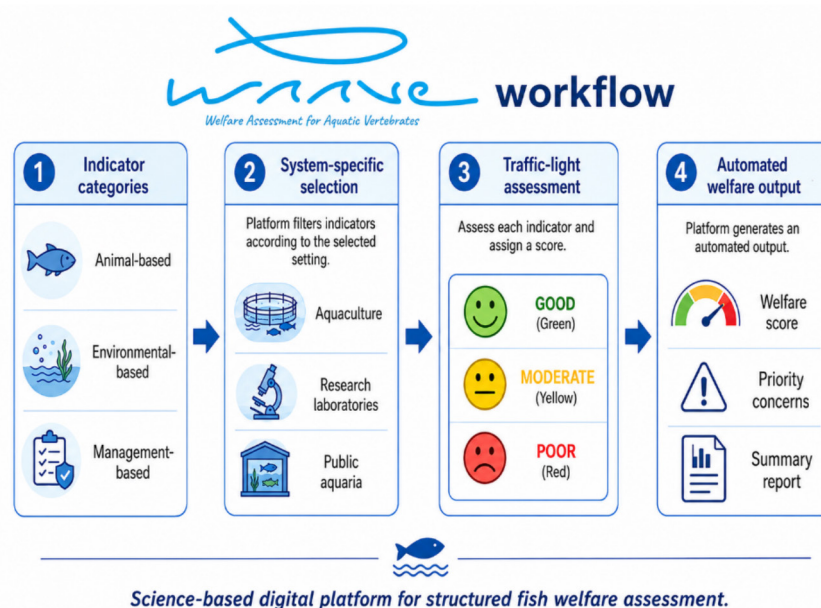


Figure 1. WAAVE workflow from indicator selection to structured welfare output.

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Scientific basis and preliminary application

WAAVE reflects work highlighting the importance of species-specific welfare knowledge (Saraiva et al., 2019), behavioural indicators (Martins et al., 2012), environmental enrichment (Arechavala-Lopez et al., 2022), and context-dependent management decisions such as stocking density (Saraiva et al., 2022). The platform has undergone internal applicability testing in FishEthoGroup facilities and preliminary use with Odysseo Oceanarium, Mauritius. These early applications suggest that a structured digital workflow can help identify welfare aspects not routinely captured through production or health monitoring alone. WAAVE is now entering a closed testing phase with selected partners to evaluate usability, indicator relevance, reporting usefulness and setting-specific applicability under operational conditions.

Discussion

WAAVE is intended to complement, not replace, existing farm management and health monitoring systems. Its contribution lies in translating welfare science into a practical, human-scored workflow that integrates behavioural, environmental and management observations. By standardizing data collection and summarizing outputs, WAAVE may support routine welfare monitoring, staff communication and continuous improvement across facilities working with fish. Future work will focus on validation and refinement of setting-specific and species-specific indicator sets.

Acknowledgements

The authors thank all collaborators and partner organizations contributing to WAAVE development, particularly Odysseo Oceanarium for its early collaboration, and the partners involved in the next testing phase. This work was co-funded by the European Union's Horizon Europe Project 101136346 EUPAHW. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

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HISTOLOGICAL ASSESSMENT OF THE GONADS OF FEMALE AFRICAN CATFISH (*Clarias gariepinus*) BROODSTOCK FED BLACK SOLDIER FLY (*Hermetia illucens*) LARVAL MEAL-SUPPLEMENTED DIETS

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Introduction

Successful aquaculture production depends on healthy and reproductively competent broodstock capable of producing high-quality gametes. Nutrition is a key determinant of reproductive performance, and increasing attention has been directed toward the use of sustainable alternative protein sources in aquafeeds. In this context, insect-derived meals have emerged as promising alternatives to conventional fishmeal. Although their effects on growth performance and fillet quality have been extensively investigated (Alfiko et al., 2022), comparatively little is known about their potential impacts on broodstock reproductive performance, gonadal development, and gamete quality. The limited studies conducted in species such as zebrafish and brown trout have reported inconsistent outcomes (Chemello et al., 2022). Consequently, the long-term implications of insect-based diets for fish reproductive health and performance remain poorly understood.

Materials and methods

Freshly hatched African catfish (*Clarias gariepinus* BURCHELL, 1822) 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* L.) (BSF) meal. 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 females from each group were selected and used for indicated propagation. After propagation fish were dissected and residual ovaries were fixed in 10% formaldehyde solution until further processing.

Sample processing was done according the protocol of Egezza et al., 2024. The prepared histological sections were examined using a Nikon Eclipse E600 research microscope. In the ovarian tissue sections, oocytes were classified according to their developmental stage, and the cells belonging to each developmental category were subsequently counted. The developmental stages of the oocytes were determined based on the classification applied by Lefler et al. (2008), according to which oocytes can be categorized into protoplasmic (prot%), cortical alveoli stage (cort%), and vitellogenic (vit%) stages.

Results

Analysis of the histological sections revealed no statistically significant differences in the distribution of ovarian oocytes across developmental stages among the dietary treatment groups. Approximately 72–74% of the oocytes were in the protoplasmic stage, while 6–7% showed the onset of cortical alveoli stage, and 19–21% were in the vitellogenic stage.

Table 1. Maturity stages of oocytes observed in the residual ovaries of individuals from the different dietary treatment groups

	prot%	cort%	vit%
Control Group	73,6%±6,6 ^a	6,9%±2,1 ^a	19,4%±7,4 ^a
BSF33 Group	72,5%±19,6 ^a	6,0%±2,1 ^a	21,5%±18,5 ^a
BSF50 Group	73,6%±5,8 ^a	6,7%±1,7 ^a	19,7%±4,1 ^a

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Discussion

The predominance of protoplasmic-stage oocytes (72,5–73,6%) and the presence of vacuolizing (6,0–6,9%) and vitellogenic oocytes (19,4–21,5%) indicate ongoing ovarian development and reproductive activity. The presence of a considerable proportion of vitellogenic oocytes suggests active yolk deposition and progression towards advanced oocyte maturation. As no significant differences were detected among dietary treatments in the distribution of oocyte developmental stages, the tested diets did not appear to adversely affect ovarian development or the progression of oocyte maturation. However, oocyte developmental stage alone does not provide a direct measure of oocyte quality or reproductive readiness and should therefore be interpreted together with reproductive performance parameters.

Conclusion

Replacing 33% and 50% of the complete feed with black soldier fly larvae meal has no negative effect on the oocyte developmental stages in the brooder age African catfish.

Acknowledgement

This research was supported by the Flagship Research Groups Programme (I.S.; Á.H.) of the Hungarian University of Agriculture and Life Sciences and NTP-SZKOLL-25-0038.

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NEUROFEED - LINKING SUSTAINABLE NUTRITION TO NEUROENDOCRINE MECHANISMS IN STRESS RESILIENCE ON EUROPEAN SEABASS: THE ROLE OF BLACK SOLDIER FLY LARVAE OIL

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Background and objective

Aquaculture intensification exposes fish to recurrent stressors, including handling, transport, vaccination and overcrowding. When stress responses are prolonged, recurrent, or severe, activation of the hypothalamus-pituitary-interrenal axis can disrupt metabolism, suppress immune competence, reduce growth and compromise welfare. There is therefore a need for affordable and sustainable feed ingredients that provide nutritional value while improving the capacity of farmed fish to cope with acute challenges. Black soldier fly (BSF) larvae oil, a co-product of *Hermetia illucens* processing, is a circular lipid source with potential functional properties. Preliminary observations in European seabass (*Dicentrarchus labrax*) indicate that dietary inclusion of this oil may attenuate the cortisol peak and improve metabolic and oxidative responses after acute stress (Basto et al., 2025). However, the neuroendocrine mechanisms underlying these effects remain unknown. This project aims to determine whether BSF larvae oil modulates central and peripheral pathways controlling stress resilience in European seabass.

Conceptual approach

The project builds on samples collected at the end of a 12-week feeding trial in which juvenile European seabass (initial body weight: 12 ± 1 g) were fed on one of two isonitrogenous, isolipidic, and isoenergetic diets: a control diet or an experimental diet containing 5.2% BSFLO, which completely replaced poultry oil. At the end of the trial, fish were sampled under basal conditions or after an acute challenge combining one minute of air exposure, 14 minutes of overcrowding at 100 kg m⁻³, and 60 minutes of recovery. The hypothalamus, pituitary gland and head kidney were collected to assess the central and peripheral coordination of the stress response. RNA sequencing of the hypothalamus and head kidney will identify diet-dependent transcriptional programmes and enriched biological pathways. Candidate genes emerging from this analysis will then be integrated with classical neuroendocrine markers and analysed by real-time quantitative polymerase chain reaction (RT-qPCR) across the three target tissues. This combined strategy will link transcriptome responses with targeted regulation of the hypothalamus-pituitary-interrenal axis.

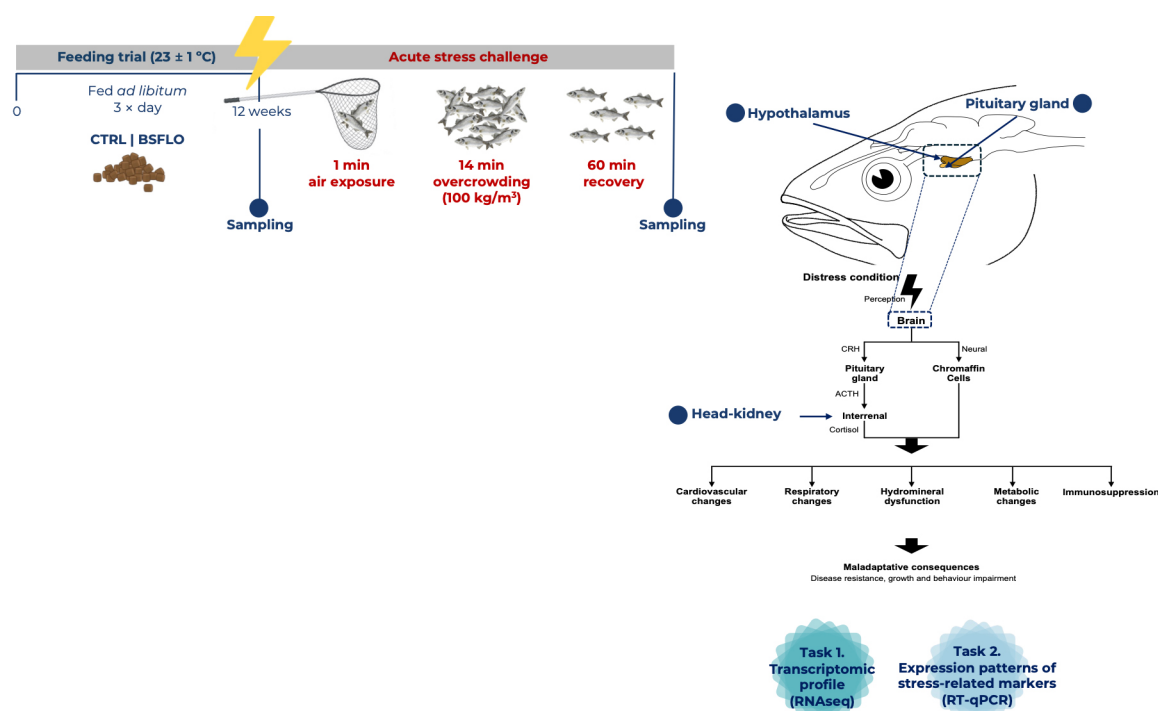


Figure 1. Conceptual overview of NEUROFEED project.

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Expected outcomes and significance

The project is expected to reveal whether BSF-derived oil reshapes the molecular response to acute stress and to identify biomarkers associated with improved resilience. By moving beyond conventional performance indicators towards a mechanistic understanding of nutrition-neuroendocrine interactions, the study will establish a biological basis for the use of BSF larvae oil as both an energy source and a functional aquafeed ingredient. The resulting knowledge may support the design of more sustainable, welfare-oriented diets while strengthening circularity through the valorisation of an insect-processing co-product.

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ALFUNFEED - FUNGAL SINGLE-CELL PROTEIN FROM MACROALGAL WASTE FERMENTATION: IMPROVING SUSTAINABILITY AND REDUCING THE CARBON FOOTPRINT OF FISH AQUAFEEDS

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Background and objective

The continued expansion of aquaculture requires sustainable, nutritionally adequate and economically viable alternatives to conventional fish- and plant-derived proteins. At the same time, macroalgal processing industries generate substantial quantities of residual biomass that remain underutilised despite containing valuable nutrients and fermentable compounds. AlFunFeed proposes an integrated circular approach in which macroalgal by-products are converted through scalable fermentation into fungal single-cell protein (SCP). By linking marine biomass valorisation with aquafeed production, the project aims to reduce waste, alleviate pressure on conventional protein resources and support the development of local circular bioeconomy value chains. The overall objective is to validate macroalgae-derived fungal SCP as a safe, functional and eco-efficient protein source for carnivorous fish species.

Conceptual approach

AlFunFeed integrates biotechnology, aquaculture nutrition and environmental assessment throughout the ingredient-development process. Selected fungal strains and fermentation conditions will be optimised to efficiently convert macroalgal residues into nutritionally valuable microbial biomass. The resulting SCP will be characterised for its proximate composition, amino acid profile, digestibility-related properties and potential safety constraints before its incorporation into nutritionally balanced experimental diets. The biological performance of the new ingredient will be evaluated in juvenile European seabass (*Dicentrarchus labrax*) through feeding trials designed to assess feed acceptance and feeding regulation, nutrient digestibility, growth performance and feed utilisation. Its potential functional effects will also be examined by evaluating immune competence, physiological condition, welfare-related indicators and gut microbial communities. This multidisciplinary assessment will determine whether fungal SCP can provide benefits beyond its role as a dietary protein source. The nutritional evaluation will be complemented by life-cycle assessment (LCA) and cost analyses comparing SCP-based feeds with conventional aquafeed formulations. These analyses will quantify potential changes in carbon and nutrient footprints, resource use and production costs, while identifying the main environmental and economic factors affecting future industrial implementation.

Expected outcomes

AlFunFeed is expected to establish a technological and biological foundation for the conversion of macroalgal by-products into high-quality aquafeed ingredients. The project will generate knowledge on production scalability, nutritional suitability, safety, functional performance and environmental competitiveness. By combining waste valorisation with the development of stable alternative protein sources, AlFunFeed may contribute to reducing aquaculture's dependence on resource-intensive ingredients and strengthening the resilience and competitiveness of European aquaculture. Engagement with researchers, industry stakeholders and policymakers will further support knowledge transfer, responsible resource use and the future adoption of circular feed-production strategies.

Acknowledgment

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REDUCING RELIANCE ON LIVE MICROALGAE IN BIVALVE HATCHERIES: POWDERED DIET SUPPORT GROWTH OF JUVENILE *Ruditapes decussatus*

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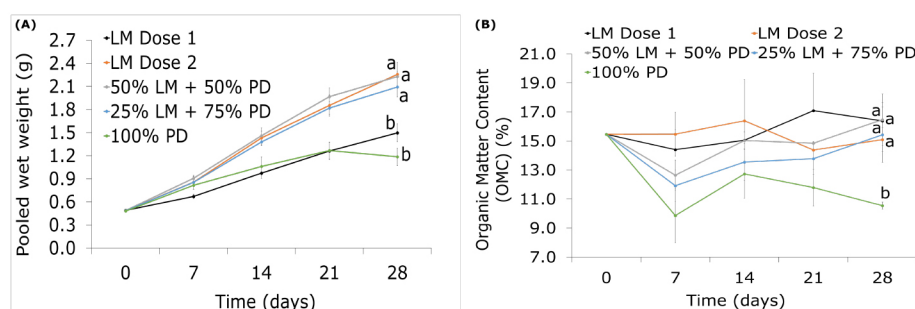
Introduction

Aquaculture production of bivalves relies heavily on the continuous supply of high-quality live microalgae¹, particularly during early juvenile stages characterized by high feeding rates and elevated nutritional demands. However, large-scale microalgae production remains technically demanding, costly, and subject to variability in both nutritional quality and availability, posing significant operational constraints for hatcheries². These limitations have driven growing interest in alternative or complementary feeding strategies capable of reducing dependence on live feeds without compromising biological performance. Among these, powdered or inert diets have emerged as promising options, offering advantages in terms of storage, handling, and consistency³. Despite these benefits, their effectiveness as partial or total substitutes for live microalgae remains insufficiently understood, particularly during juvenile development. This knowledge gap is especially relevant for high-value species such as *Ruditapes decussatus*, in which early-stage nutrition strongly influences growth performance and overall physiological condition⁴. Therefore, this study aimed to evaluate the effects of partial and total replacement of live microalgae with a formulated powdered diet (PearlDiet) on the growth and physiological condition of juvenile *R. decussatus*.

Materials and Methods

Juvenile *R. decussatus* (~1 mm initial shell length) were reared for 28 days in 8 L closed systems using ultrafiltered seawater. Clams were distributed as pooled samples (~0.5 g wet weight, WW) across five dietary treatments in triplicate: 1) live microalgae (LM) Dose 1; 2) LM, Dose 2; 3) 50% LM + 50% PearlDiet (PD); 4) 25% LM + 75% PD; and 5) 100% PD. LM consisted of *Tisochrysis lutea*, *Chaetoceros calcitrans*, and *Skeletonema costatum* (1:1:1, v/v), while PD comprised a microalgal blend fortified with docosahexaenoic acid (DHA) and supplemented with carbohydrates, probiotics, and yeast. Diets were administered daily in two feeding events at fixed rations: LM at 10% (Dose 1) and 5% (Dose 2) and PD at 15% (g dry weight g⁻¹ WW of juveniles). Water was renewed every two days and continuous aeration was provided. Environmental conditions were monitored daily. Sampling was performed weekly, with biomass standardized at each time point to maintain comparable system density. Growth was assessed based on changes in WW and shell morphometrics (shell length, SL; shell height, SH; shell area, SA), while physiological condition was evaluated through organic matter content (OMC).

Figure 1.



(Continued on next page)

Results

At the end of the experiment, there were no significant differences in WW, SL, or SA among juveniles fed all LM-containing diets, except for those fed LM Dose 1, which showed the lowest growth (WW, SL, and SA) among these treatments. The growth of juveniles fed 25% LM + 75% PD (WW: 2.09 ± 0.13 g, SL: 3.51 ± 0.82 mm, SA: 7.17 ± 2.9 mm²) did not differ significantly from that of juveniles fed LM Dose 2 (WW: 2.26 ± 0.16 g, SL: 3.68 ± 0.94 mm, SA: 7.33 ± 3.11 mm²). Juveniles fed 100% PD showed the lowest WW, SL, SA, and OMC values, which were significantly lower than those observed in most dietary treatments. No significant differences in OMC were observed between juveniles fed 25% LM + 75% PD and those fed LM Dose 2 or any other LM-containing diets. Juveniles fed 100% PD showed significantly lower OMC values ($10.55 \pm 0.25\%$) compared to all other treatments.

Growth performance and physiological condition of juvenile *Ruditapes decussatus* over a 28-day feeding trial under different dietary treatments. (A) pooled wet weight (g); (B) organic matter content (OMC, %). Values are presented as mean $\pm$ SD ($n = 3$). Different lowercase letters indicate significant differences among treatments at day 28 (one-way ANOVA, Tukey post-hoc test, $p < 0.05$). LM: live microalgae; PD: PearlDiet

Discussion

The present study demonstrates that a substantial reduction in live microalgae supply is possible without compromising the growth and physiological condition of juvenile *R. decussatus*. Comparable growth and OMC between the 25% LM + 75% PD and LM treatments, particularly relative to LM Dose 2 (the best-performing LM treatment), indicate that a substantial proportion of live microalgae can be replaced by a formulated powdered diet while maintaining juvenile clam performance. This finding is especially relevant, as live microalgae constitute the natural diet of bivalves and their production remains a major technical and economic constraint for bivalve seed production¹. Similar approaches have been reported in bivalve spat, where non-live or concentrated microalgal diets have been shown to support growth when used as complementary feeds rather than as complete substitutes³. The lower performance observed in the 100% PD treatment suggests that further improvements in formulation are needed to fully match the nutritional and functional properties of live microalgae. However, the strong performance observed at high inclusion levels of inert diet highlights the potential of partial replacement strategies to improve hatchery efficiency. Overall, these findings support the use of PearlDiet as a complementary feed to reduce reliance on live microalgae, thereby enhancing production sustainability and reducing operational costs.

Acknowledgment

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OPTIMIZING ELECTRICAL STUNNING OF COMMON CARP: STUNNING EFFECTIVENESS, WELFARE AND FILLET QUALITY PERSPECTIVE

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Introduction

Common carp (*Cyprinus carpio*) is one of the most widely cultured and consumed fish species in Central Europe. Ensuring humane slaughter is essential not only for fish welfare but also for maintaining fillet quality. However, many conventional slaughter methods do not consistently meet fish welfare standards. Electrical stunning (ES) has emerged as a promising humane slaughter technique, as species-specific studies indicate that it can induce an irreversible stun or a reversible state of unconsciousness. Nevertheless, if ES is applied improperly, it may compromise both fish welfare and product quality. In common carp, the effectiveness of ES depends strongly on the optimization of electrical parameters. Therefore, precise control and validation of these parameters are critical to ensure reliable induction of unconsciousness while preserving fillet quality. This study is designed to optimize electrical stunning conditions at an industrial scale, with the aim of improving both welfare outcomes and product quality in common carp processing.

Materials and Methods

The in-water electrical stunning (ES) protocol was evaluated in common carp (3–4 kg) at a commercial fish processing plant (Rybářství Chlumec nad Cidlinou, Czech Republic). Two stunning systems were used: an existing plant-operated stunner (AC, 230 V, 50 Hz, 5 A) and a newly developed ES unit capable of both AC and DC application (50 Hz, adjustable 0–320 V and 0–15 A). A total of 80 fish were randomly assigned to four treatment groups (n = 20 per group): percussion stunning as the control (T0), T1 (commercial stunner; AC, 220 V, 5 A, 7 min), T2 (AC, 320 V, 6 A, 5 min), and T3 (DC, 320 V, 6 A, 5 min).

To assess the effectiveness of stunning, immediately after stunning, 10 fish per treatment were transferred to a recovery tank to monitor the return of consciousness at 0, 2.5, 5, 7.5, and 10 minutes post-stunning. Physiological stress responses were evaluated using blood indicators (cortisol, lactate dehydrogenase, glucose, potassium). For the product quality assessment, rigor mortis progression and muscle pH were monitored over 72 hours (0, 6, 12, 24, 36, 48, 60, and 72 h). Additionally, key fillet quality parameters such as drip loss, ATP, glycogen, glucose, lactate, haemoglobin content, color, and texture were analyzed using standard protocols.

Results

Fish subjected to percussion and the newly developed DC stunning (T3) did not regain consciousness during the 10-minute post-stunning observation period, indicating a prolonged unconsciousness and effective stunning. In contrast, 50% of the fish treated with the plant stunner (T1: AC) regained consciousness within 10 minutes, while an even higher recovery rate (70%) was observed in the T2 (AC) group, suggesting less effective stunning. Cortisol and other blood parameters did not differ significantly ($p > 0.05$) among treatments compared to percussion, indicating a comparable acute stress response across groups. However, clear differences were observed in post-mortem muscle dynamics and fillet quality. The onset of rigor mortis occurred significantly earlier ($p < 0.05$) in T1 (at 12 h), followed by T2, T3, and finally T0 (percussion), suggesting accelerated metabolic exhaustion in AC-treated fish. The pH of fish muscle is also consistent with rigor progression. Drip loss was slightly higher ($p > 0.05$) in all electrically stunned groups compared to percussion, indicating reduced water-holding capacity. Muscle glycogen content was significantly lower in T1 and T2, but higher in T0 and T3 ($p < 0.05$), reflecting greater pre-mortem energy depletion in AC treatments. In contrast, glucose levels were highest in T1, consistent with increased glycogen breakdown. Additionally, muscle haemoglobin content was significantly elevated in T1 compared to other treatments ($p < 0.001$), suggesting greater tissue damage or blood retention. Overall, these results indicate that DC (T3) stunning provides effective induction of prolonged unconsciousness comparable to percussion, while preserving better fillet quality than AC (T1 & T2) treatments.

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SUBMERGED FARMING AS PREVENTIVE MEASURE AGAINST *Lepeophtheirus salmonis*: THE IMPORTANCE OF PARASITE BIOLOGY AND BEHAVIOUR KNOWLEDGE

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Lepeophtheirus salmonis infestation is a major challenge for the Atlantic salmon (*Salmo salar*) aquaculture industry. Submerged farming (sea cages below 20 m depth) has emerged as a promising preventive measure against the parasite, being based on that there is a higher abundance of infective stages of *L. salmonis* in upper water layers. Although infestation levels seem reduced, some infestation still occur in submerged cages, and infestation mechanisms are poorly known. The current study summarises several investigations of *L. salmonis* biology and behaviour related to submerged farming.

Production data from submerged farms were collected and analysed. Different life stages of *L. salmonis*, including egg strings, were collected from submerged cages and brought to the laboratory. Survival was investigated, and hatching and development of larvae from the egg strings was monitored. Behavioural responses of nauplii, copepodites, pre-adult and adult stages to stimuli such as light, pressure, and different salinities were quantified.

In the current data set, there was a 90% reduction in delousing treatments in submerged cages compared to conventional surface cages, highlighting the positive effect of submerged farms but showing that infestations still happen. Survival of *L. salmonis* collected at submerged farms did not differ from that of lice from surface farms, neither did hatching and development of larval stages. This indicates that the depth of submerged cages does not represent a challenge to the basic biology of the parasite. Behavioural experiments with larvae originating from submerged and surface farms show that the larvae are positively phototactic and negatively barotactic, and avoid certain salinities. However, light, pressure, and salinity mediated behaviour differently depending on *L. salmonis* origin. The results highlight the need for detailed knowledge on biology and behaviour of the parasite to predict the effects of preventive measures, and that this knowledge is needed to optimise the measures.

LINKING GENOMICS AND SHELL MORPHOLOGY IN MANILA CLAM (*Ruditapes philippinarum*): INSIGHTS FROM SNP ARRAYS AND CT IMAGING

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Background

The Manila clam (*Ruditapes philippinarum*) is one of the most widely cultivated bivalve species globally, valued for its rapid growth, environmental tolerance, and economic importance in coastal aquaculture. Its production is increasingly challenged by disease outbreaks, rising temperatures, and other environmental stressors linked to climate change. Selective breeding, supported by genomic tools, offers a powerful approach to enhance resilience, productivity, and key commercial traits in this species.

Previously, we have developed and validated a high-density SNP array for the Manila clam to enable large-scale genomic analyses supporting selective breeding. SNP discovery was conducted using low-coverage whole-genome sequencing across multiple European populations, followed by the design and validation of a 49K Axiom® SNP array. The platform demonstrated high call rates and robustness across diverse experimental cohorts, providing a comprehensive resource for downstream analyses of heritable traits, including growth, disease resistance, and thermal tolerance.

Methods

To complement genomic data, a large-scale phenotyping effort was undertaken using computed tomography (CT) scanning of 1000 clam shells. A purpose-built holder was designed and iteratively refined to ensure consistent positioning and optimal scan quality. High-resolution scans were used to extract detailed morphometric parameters, including shell width, depth, thickness, and density, enabling precise quantitative characterization of shell structure.

Results

The CT-derived data provides a novel, high-resolution characterization of shell morphology and allows deeper investigation into which morphological traits are most strongly influenced by genetic variation, strengthening the integration of phenotypic and genomic datasets for selective breeding applications.

INTEGRATED MULTI-OMICS OF MICROBIAL COMMUNITY STRUCTURE OF RAINBOW TROUT GUT AND CULTURE SYSTEM

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Introduction

The assembly of the gut microbiome in fish is a dynamic and tightly regulated process influenced by host genetics, early-life environmental exposure, diet, and aquaculture practices. Although, the host-focused RNA-seq datasets are routinely generated in aquaculture research and breeding programs yet the microbial information contained in unmapped reads is typically overlooked. Advances in high-throughput sequencing technologies, along with the multi-omics approaches has enabled integration of microbial community structure with the functional characterization. This combined approach provides insights into microbial roles and will improve understanding host-microbiome-environment interactions and aquaculture sustainability. This study aims to comprehensively characterize the intestinal meta-transcriptome of *Oncorhynchus mykiss* using different rearing systems, with particular emphasis on the influence of early-life rearing environments.

Materials and Methods

Eyed eggs (200 degree-days post fertilization) of specific pathogen-free (SPF) rainbow trout (*Oncorhynchus mykiss*) were reared in two contrasting systems flow through system (FTS: low organic matter) and Recirculatory Aquaculture System (RAS: high organic matter), each in triplicates. At late fry stage (35 days post hatching, dph) the gut samples were collected along with corresponding water microbiome samples via vacuum filtration using membrane filters (0.22 µm nitrocellulose membrane filter, 47 mm diameter, Millipore™). Total DNA and RNA was extracted from both the sample types. Microbial community composition will be characterized using 16S rRNA gene amplicon sequencing to study the influence of rearing conditions on the microbiome assembly. Furthermore, meta-transcriptomic analysis of gut-associated microbial communities and water samples was conducted by RNA-seq datasets. These poly(A)-selected RNA-seq enriched for eukaryotic mRNA, enable retrospective investigation of microbial functional dynamics under various baseline conditions. Relevant datasets will be identified using targeted keywords including “rainbow trout” and “*Oncorhynchus mykiss*”, “fish gut microbiota”, “microbial dysbiosis fish” along with the study type “gut meta-transcriptome”. Paired-end reads will be processed using standardized bioinformatics pipelines incorporating automatic library type detection, quality control, and validation of read mappings.

Expected Results

It is expected that microbial community in the two rearing systems vary significantly in both water and gut. The RAS with more stable water microbiota will support a more stable microbiota with higher alpha diversities. In contrast, the FTS with continuous water exchange harbor less stable microbial community often dominated by potentially pathogenic r-strategists indicating, including opportunistic or pathogenic taxa. These differences in environmental microbial composition are expected to directly influence initial gut colonization and subsequent microbiome assembly in rainbow trout.

Discussion

Early-life rearing conditions are known to significantly influence the microbial community assembly in both water and gut environments of fish. FTS and RAS previously showed differences in the water microbial community due to the differences in water exchange. In addition, the bacterial density in water entering the rearing tanks of RAS was shown to be more than 10 times higher than in the FTS. It is expected that early environmental microbial conditions will have lasting impacts on both microbial functional potential and host intestinal health.

Overall, it is anticipated that aquaculture system design plays a central role in shaping microbiome- development and resilience in fish, supporting the concept that early microbial environments have long-term implications for aquaculture and productivity.

Acknowledgement

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IDENTIFICATION AND MAPPING OF SUITABLE SITES FOR GILTHEAD SEABREAM FARMING IN THE MEDITERRANEAN: INTEGRATION OF OCEANOGRAPHIC PARAMETERS, DIETARY REGIMES, FUNCTIONAL FEED FORMULATION, INTEGRATED MULTI-TROPHIC AQUACULTURE, AND PARASITIC PRESSURE

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Mediterranean coastlines face escalating pressures from urbanization, anthropogenic pollution, and climate change impacts, making the identification of viable sites for marine aquaculture a major scientific and decision-making priority. Gilthead seabream (*sparus aurata*) is an ecologically and economically important species for Mediterranean regions, but it presents strict environmental requirements regarding temperature, salinity, hydrological dynamics, water quality, trophic availability, and parasitic exposure. These parameters vary across coastal sectors, necessitating an integrated spatial approach to optimize farm site selection and adapt nutritional formulations and sustainable production strategies accordingly.

This study evaluates the suitability of Mediterranean coastlines for gilthead seabream farming by leveraging oceanographic and biogeochemical variables from the Copernicus program (Copernicus, 2023), particularly sea surface temperature, salinity, marine currents, and chlorophyll-a concentration. The analysis aims to identify coastal zones offering optimal conditions for marine farm establishment, while accounting for major ecological constraints including coastal pollution, ecosystem fragility, and seasonal environmental variability. Copernicus data will be systematically validated and corrected through advanced quality control procedures. Python will serve as the primary analysis tool with specialized libraries for geospatial and statistical processing.

Beyond physicochemical parameters, this study integrates critical biological factors: the natural dietary regime of *sparus aurata*, its physiological adaptation to seasonal variations in trophic resources, and its susceptibility to parasitic pressures (Koven, 2002). These elements directly influence farming performance and guide the formulation of functional feeds tailored to each geographic zone. To enhance sustainability and production resilience, this research explores a multi-trophic aquaculture approach (Chopin, 2013) combining gilthead seabream and European sea bass farming with the culture of mussels (*mytilus edulis*) and oysters (*crassostrea gigas*). This strategy leverages ecological relationships based on mutualism and commensalism, where fish waste becomes nutritional resources for filter-feeding mollusks, which simultaneously provide biological purification and naturally regulate dissolved oxygen, pH, and ammonia accumulation.

The integration of all these factors (oceanographic parameters, biological characteristics, nutritional requirements, multi-trophic systems, and parasitic pressure assessments) will produce comprehensive spatial suitability mapping and deliver an evidence-based framework for sustainable aquaculture planning adapted to Mediterranean coastal realities. This holistic approach reconciles aquaculture development with coastal ecosystem preservation, optimizes production performance through location-specific functional feed formulations, and enhances environmental stability through integrated multi-trophic aquaculture systems.

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CRUDE ALGAL EXTRACTS FOR ENHANCED BIOMASS PRODUCTION IN DIGESTATE-FED MICROALGAL CULTURES

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Introduction

Microalgae cultivation on wastewater and anaerobic digestate enables simultaneous biomass production and nutrient recovery while reducing the need for mineral fertilisers. The resulting biomass can be valorised as biostimulants, biofertilisers, feed ingredients, bio-based materials, or energy products (Berden-Zrimec *et al.*, 2024). However, biomass productivity in digestate-fed systems is often lower than expected from nutrient availability alone. Previous studies have shown that additional CO₂ supplementation can significantly improve algal growth, indicating that inorganic carbon availability may become limiting under cultivation conditions (Berden-Zrimec *et al.*, 2026). While effective, continuous CO₂ injection increases operational costs.

Recent laboratory studies demonstrated that crude algal extracts prepared from *Scenedesmus*-dominated biomass stimulate growth in digestate-based cultures, suggesting improved utilisation of inorganic carbon already present in the medium (Berden-Zrimec *et al.*, 2026). The present study evaluated whether a similar effect could be achieved in pilot-scale digestate-fed raceway ponds.

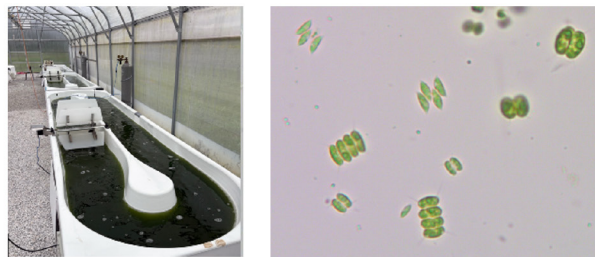


Figure 1. Raceway pond culture fed with anaerobic digestate and dominated by *Scenedesmus dimorphus* and *S. quadricauda*.

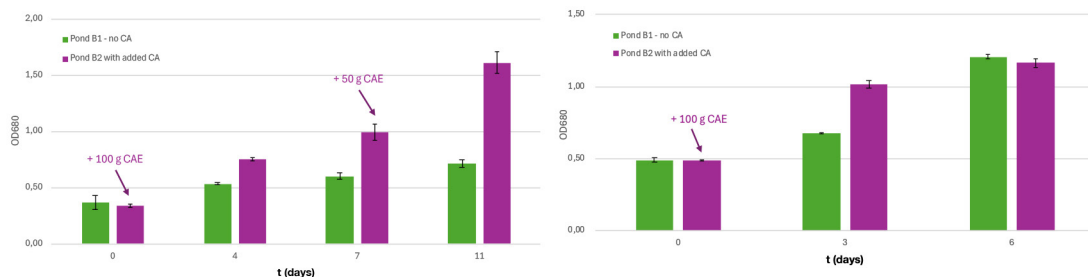


Figure 2. Experiments results: (left) First study, (right) Second study.

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Materials and methods

Experiments were conducted in identical raceway ponds (7.5 m², approximately 1250 L) cultivated on biochar-filtered anaerobic digestate from a food-waste biogas plant (Ljubljana, Slovenia). The ponds (Figure 1) were operated under the same conditions with digestate addition of approximately 10 L da⁻¹ and automatic pH control through CO₂ injection. The dominant species were *Scenedesmus dimorphus* and *S. quadricauda* (Figure 1).

Crude algal extract (CAE) was prepared from harvested pond biomass by drying and mechanical disruption and was directly added to the selected pond (100 g). Biomass growth was monitored by optical density (OD₆₈₀) and dry weight measurements. Two approaches for lipid stimulation were applied after the growth phase to explore the possibility of digestate-grown biomass valorisation for fuels.

Results and discussion

CAE supplementation increased biomass accumulation in both pilot-scale experiments (Figure 2). After 11 days of the first trial, the CAE-treated pond reached more than twice the biomass concentration of the untreated control. The first experiment was affected by intermittent power outages and interrupted digestate feeding between days 4 and 6. Despite these disturbances, the CAE-treated pond maintained substantially higher biomass accumulation than the control, suggesting improved culture robustness under suboptimal cultivation conditions. The second experiment was performed later in the season (Figure 2b) in stable operation conditions, exhibiting difference between treatments only in the first days after supplementation.

The lipids production was not stimulated by lowering the digestate addition and adding phosphate. On the other hand, removing 75% of the medium and replacing it with water with phosphate, resulted in alteration of the composition of total lipids content. Although the total lipids were rather constant, their composition in phospholipids was decreased suggesting an enrichment of total lipids with glycerides. The free fatty acids produced by the degradation of phospholipids can be utilized for the synthesis of glycerides.

The findings indicate that the stimulatory effect of CAE is reproducible and pronounced shortly after addition. While CAE initially stimulated biomass accumulation in both experiments, the effect was stronger and more persistent under interrupted feeding and other suboptimal cultivation conditions. CAE supplementation may represent a practical tool for improving biomass productivity in digestate-fed cultures by enhancing the utilisation of nutrients and inorganic carbon already available in the cultivation medium.

Acknowledgements

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OLAMUR – LARGE SCALE REALISATIONS OF MULTI-USE SCENARIOS WITH LOW-TROPHIC AQUACULTURE

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Introduction

OLAMUR Offshore Low Trophic Aquaculture in large Scale Realisation is a large EU Flagship project in the Baltic/North Sea region (2023-2026) of the Mission Ocean and Waters. The project comprises 25 partners in 8 countries, with three major case studies (Figure 1). Since 2025, five Associated Regions have been attached to the project, in Ireland, Spain, Portugal, Türkiye, and Bulgaria, expanding the project's activity to the Atlantic, the Mediterranean and the Black Sea. The overall aim is to explore and enhance concepts of multi-use of marine space. The purpose of this presentation is to provide a brief overview of the project's activities many of which will be presented in the session on "offshore and multi-use" at the Aquaculture Europe 2026.

Results and tentative conclusions

LTA cultivation experiments with seaweeds and mussels have been carried out in all three case studies, and results are being analysed. In addition to growth yield, analyses of contaminants are being processed. Both the Baltic and the North Sea are characterized by extensive human activities, shipping, fisheries, and surrounded by populated shores. Especially the Baltic are receiving excess nutrients from waterways draining into the basin. The Baltic, which is only connected to the North Sea through the narrow and shallow Danish straits, is also characterized by low salinity, particularly in the inner parts.

Growth yield of seaweeds were strongly related to local conditions, of which salinity plays a major role. This is in accordance with estimates models published early in the project (Maar et al. 2023). In the Estonian case, the seaweed that was cultivated was *Ulva* sp, which tolerates low salinity much better than the *Saccharina* sp cultivated at site A and B. With blue mussels, good yields were obtained at all sites.

Stakeholder interviews were carried out involving LTA farmers, wind farmer industry and legislators in the different countries. Legislation is highly variable among European nations, and even among German Länder. Whereas some countries (Estonia) have developed legislation taking into account that LTA may neutralize excess nutrients from other activities, most countries practice the sector principle, which *de facto* counteracts multi-use concepts.

LTA farming is at different stages of development. In general shellfish have better developed markets than farmed seaweeds. Upscaling is a prerequisite to obtain sustainable industries. Synergies were obtained between the different industries, in particular with vessel operation and with surveillance of the utilized area. The five associated regions are at different stages with respect to development of the multi-use concepts. LTA is carried out in most countries, whereas the Portuguese case includes a floating offshore wind farm.

The main conclusion is that large-scale multi-use of area is possible and is an attractive pathway to obtain more area-efficient utilization of European marine area. We have also found it advisable to include the different industries in such a way that multi-use is beneficial for all partners.

Acknowledgments

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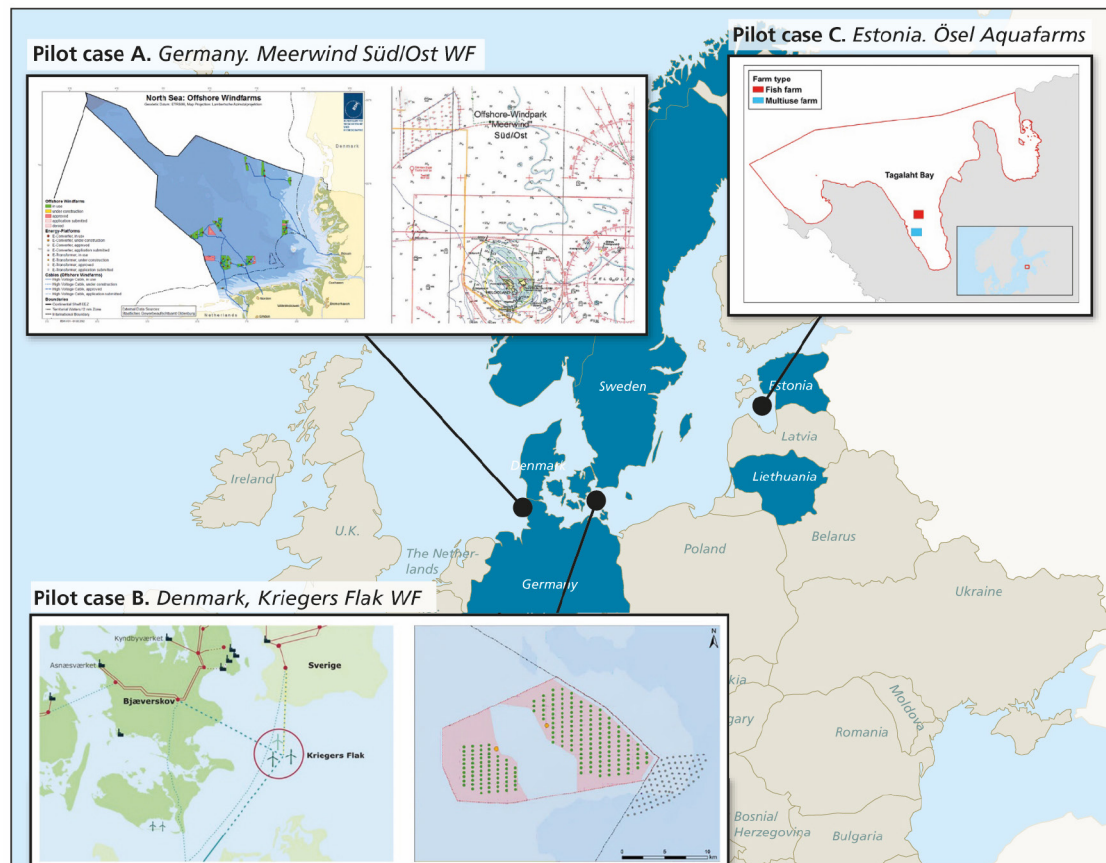


Figure 1. Case studies of the OLAMUR project. Case A and B combines LTA with wind farms, whereas Case C combines LTA with a rainbow trout farm.

EXAMINATION OF THE CULTURE OF INNOVATION, RESOURCE UTILIZATION, AND PROJECT MANAGEMENT IN THE HUNGARIAN AQUACULTURE SECTOR

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Introduction

Aquaculture is one of the world's most dynamically developing agricultural sectors (Cai & Leung, 2024; Mair et al., 2023; Verdegem et al., 2023). Methodological advancements, the development of new technologies, product development, digitalization, and the preservation of biodiversity are all cornerstones of the European Green Deal formulated by the European Union. Innovation and research and development are the driving forces behind these processes (COM, 2020; FFS, 2021).

In our study, we sought to assess the grant application activities of the Hungarian aquaculture sector within the framework of three main themes (innovation, resource utilization, and project management).

Materials and Methods

In compiling the questionnaire, we formulated 17 strategic questions covering several major themes that addressed the core issues of the research. In the first group, we examined the general characteristics of the businesses, such as their nature, duration of active operation, and size. In the next thematic area, we examined the companies' history of grant applications, their current status, their objectives (e.g., energy investments, innovation, digitalization, research and development of ecosystem services, etc.), and their opinions regarding the difficulties encountered in connection with grant applications. In the final topic group, the questions focused on companies' opportunities for obtaining information related to projects, as well as their experiences with project management.

Results and Discussion

Based on our survey, the Hungarian aquaculture sector is open to implementing research, development, and innovative investments, and has recognized the importance of collaborating with universities and research institutes (Mankins, 1995). However, the importance of digitalization, organizational development, and human resource development must be emphasized. The main constraints in terms of grant application activity are the administrative burden and the high proportion of own contribution required under the criteria (EC, 2021; R, 1380/2013). The European Union's new guidelines (initiatives) and public consultations may offer solutions to these current problems. Innovation in the accessibility of grant calls is unavoidable; the involvement of new platforms is crucial (HUNATiP, 2018). The sector possesses both empirical and theoretical project management experience. Applications are managed both within companies and with the involvement of external experts (companies).

The topics covered by the exploratory questionnaire can be expanded to facilitate more in-depth and comprehensive research. It is also important to engage additional segments of the aquaculture sector (fishmongers, feed manufacturers).

(Continued on next page)

Acknowledgment

We would like to thank all representatives of the Hungarian aquaculture sector for contributing their insights to these results.

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THE POTENTIAL OF PROBIOTIC SUPPLEMENTATION FOR IMPROVED GROWTH, SURVIVAL AND RESILIENCE OF BALTIC SALMON *Salmo salar* AND PIKEPERCH *Sander lucioperca*

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Introduction

Baltic salmon (*Salmo salar*) and pikeperch (*Sander lucioperca*) hold paramount economic and ecological importance to the Baltic region. Despite their value, these fish exhibit significant sensitivity to environmental stressors, including pollution and fluctuating water quality parameters. Currently, their biological fitness and long-term resilience are increasingly threatened by anthropogenic pressures, climate change, and the prevalence of diseases in hatchery environments (Veedu & Thomas, 2026). This vulnerability often leads to an unsustainable reliance on antimicrobial agents, which compromises the quality of fish rearing and contributes to the growth of antimicrobial resistance (Merrifield *et al.*, 2010). To address these challenges, the present study investigated the efficacy of probiotic supplementation, aiming to evaluate its impact on fish growth, survival, and health across various developmental stages (Imentai *et al.*, 2020).

Materials and methods

Experimental trials on juveniles were conducted within two different rearing environments, specifically, flow-through system (FTS) and recirculating aquaculture systems (RAS) (Table 1). This approach aligns with prevailing regional cultivation practices and ensures the evaluation of results under varying conditions. Additionally, trials with Baltic salmon compared two distinct probiotic administration routes: dietary supplementation via feed and immersion baths. Tested commercially produced probiotics consisted of a synergistic blend of active ingredients: lactic acid bacteria (*Lactobacillus* spp., *Sporolactobacillus* spp., and *Weizmannia* spp.), yeast cultures, fermented plant and herb extracts, phyto-ferments, sugar cane molasses, natural minerals and sea salt.

Results and discussion

Research outcomes demonstrate that the effectiveness of probiotic treatment is highly dependent on both the developmental stage of the fish and the delivery method.

1. Early-stage Baltic salmon (fry and early parr): bath immersion showed more favourable outcomes than dietary supplementation. Key benefits included enhanced survival rates, stabilized feeding behaviour, and a marked reduction in physiological stress symptoms.
2. Pathology mitigation: in Baltic salmon parr specifically, bath applications resulted in a 20% average reduction in the incidence of gill cover necrosis.
3. Late-stage salmon (presmolts): for fish at the presmolt stage, the dietary route became the most effective, leading to significant improvements in measurable growth metrics.
4. Pikeperch fry: long-term dietary intervention proved most impactful. A three-month probiotic feeding regimen administered during the peak growth period resulted in a substantial increase in both body weight and condition factor.

Table 1. Experimental trials conducted on salmon and pikeperch juveniles.

System type/fish species	Baltic salmon			Pikeperch	
Flow-through system	Fry	Parr	Presmolts	-	-
RAS	-	Parr	Presmolts	Fry (4-6 months)	Older fingerlings (6+ months)

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These findings confirm that the efficacy of probiotic application is highly stage- and species-specific. Consequently, optimizing delivery strategies — transitioning from immersion baths in early life stages to targeted dietary supplementation during peak growth periods — represents a promising approach to improving fish health and resilience across aquaculture operations.

By providing a targeted means to support fish health and development, probiotics may represent a sustainable preventive strategy that could help reduce the need for antimicrobial treatments. Such an approach may contribute to addressing the growing challenge of antibacterial resistance. Ultimately, the integration of species-specific probiotic protocols may contribute to the long-term viability and ecological fitness of Baltic salmon and pikeperch resources, while supporting more robust and sustainable hatchery production. This strategy not only enhances immediate growth outcomes but may also contribute to improved fish health and resilience, supporting a more sustainable approach to modern aquaculture.

Acknowledgment

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IMPROVEMENT OF NON-INVASIVE PREDICTION OF CARCASS YIELD IN FISH USING AN AI-DRIVEN PHENOTYPING PIPELINE COMBINING ULTRASOUND AND 2D/3D IMAGING

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Introduction

Improving carcass yield through breeding program is essential to improve the economic and environmental sustainability of fish farming, particularly for species marketed as processed fillets, such as rainbow trout or salmon. Hence, predicting carcass yield accurately on breeding candidates is key to maximize the genetic gain achievable for this trait. The method currently used to predict carcass yield on candidates is to measure, using ultrasound, the thickness of the fillet below the ribs (E8) and the total depth of the belly cavity (E23) (Haffray *et al.*, 2013). With those metrics, breeders are able to predict viscero-somatic index and thus carcass yield. The R^2 between the ratio E8/E23 and carcass yield is about 0.3. The aim of the present study was to improve this prediction and to facilitate the phenotyping of carcass yield on candidates by developing a phenotyping machine combining ultrasound with 2D and 3D images analysed by an AI pipeline.

Materials and Methods

We developed an integrated phenotyping machine combining a 2D camera (Keyence VS Series), a 3D camera (Zivid 2+ MR60) and an ultrasound (IMV Exapad). In parallel, we developed an AI framework (FishFusionNet) capable to analyse the different information provided by these devices.

Prior to being processed by the FishFusionNet, the data from each device underwent specific preprocessing steps. For the 2D images, we developed two YOLO-based models: one to automatically detect 15 anatomical keypoints at strategic locations on the fish (FishKpt), and another to segment the different body parts (FishSeg). These segmentations were then used in combination with the 3D data to estimate volumes of the respective body parts. The 3D acquisitions also enabled the generation of a surface heat map of the fish.

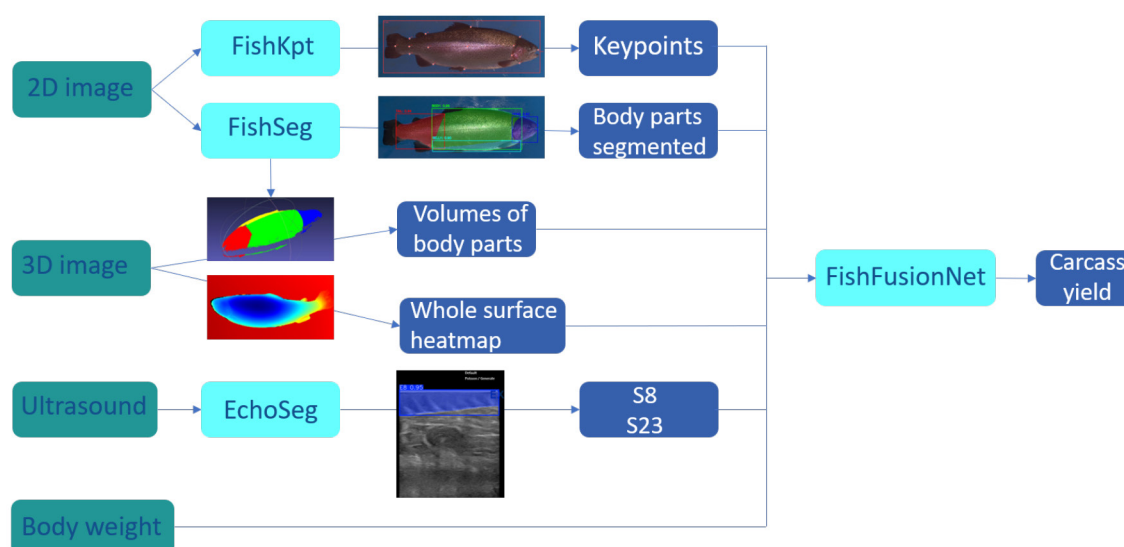


Figure 1. Pipeline of the FishFusionNet AI model.

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Finally, we developed YOLO-based models (EchoSeg) to segment E8 and E23 features on ultrasound images. To calibrate and validate the different models for rainbow trout, we phenotyped two different populations of fish derived respectively from Viviers de Sarrance (960 fish) and Bretagne Truite (1230 fish) nucleus.

Results and Discussion

The initial results of the FishFusionNet, trained on data from Viviers de Sarrance, show a substantial improvement of the prediction accuracy compared to the traditional ultrasound method, with an R^2 of 0.62 between the carcass yield measured and the carcass yield predicted. However, these results do not yet include ultrasound data, as the EchoSeg model is currently under development. We therefore expect further gains in predictive performance with the integration of ultrasound features via EchoSeg, as well as through the inclusion of the data from the fish of Bretagne Truite. Then, since fish from both populations were genotyped, we will estimate the genomic correlation between the measured carcass yield and the predicted carcass yield. This is to verify whether predicted carcass yield represents the same genetic trait as the measured carcass yield.

Acknowledgment

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TESTING LONG-TERM CRYOPRESERVATION OF BLUE SHRIMP (*Litopenaeus stylirostris*) SPERMATOPHORES

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Context and Methodology

The blue shrimp was introduced in the 70s in Hawaiï, New-Caledonia and French Polynesia leading currently to some breeding programs (Haffray et al., 2025). The long-term cryopreservation of the male genetic resource would be an interesting backup. Thus, this study aimed at testing the methodology used to cryopreserve the banana shrimp (*F. merguiensis*) spermatophores (Nimrat et al., 2022).

To do so, 118 mature males were sampled either in cryovials (the whole spermatophore ; n=140) or in straws (a suspension of the sperm mass). The fresh control consisted of the 20 males used for straw cryopreservation. The extender was the same calcium-free saline solution as in Nimrat et al. (2022), adding antibiotics (streptomycin-penicilin) and DMSO (dimethylsulfoxide) as cryoprotectant. There were 20 min of equilibration prior to freezing on a floating rack 3 cm above liquid nitrogen for 10 min before storing in the liquid nitrogen tank. Viability controls (eosine staining under light microscopy) happened just after freezing, then one, two, three, five and seven months later. Thawing lasted 20 s for straws and 160 s for spermatophores at 30°C.

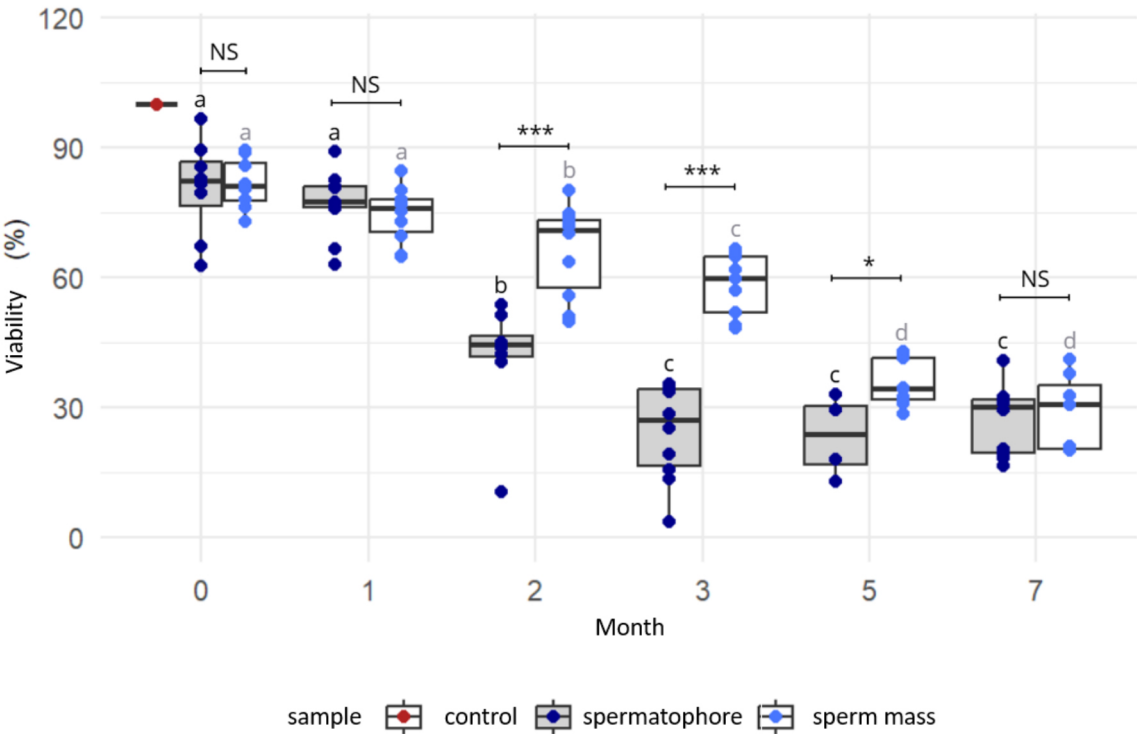


Figure 1: Assessment of sperm viability during cryopreservation either in cryovials (spermatophore) or in straws (sperm mass)

(Continued on next page)

Results and Disucssion

The Viability in the fresh controls was 100%. The viability of the cryopreserved samples lowered over the eight-month storage in liquid nitrogen from 80% to 27%. The decrease was slower in straws than in cryovials (Figure 1). However, the fertilization success is the ultimate test to ensure that cryopreservation can work as a backup for the genetic resources of the species. A pre-test was conducted with 6 mature females that were manually fertilized with 15 thawed spermatophores and several hundreds of larvae were obtained.

These preliminary results are the first mentioned in blue shrimp. It was a proof-of-concept but there is still room for methodology improvements so that the viability remains higher for a longer time. The most important being to ensure good storage conditions and regular supply in liquid nitrogen (a rise in temperature inside the tank is the strongest hypothesis to explain any decline in viability). The next step would be to apply a dedicated design for manual fertilization from thawed material to truly validate the whole process.

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PALATABILITY OF BLACK SOLDIER FLY LARVAL MEAL IN *Penaeus vannamei*: A BIOCHEMICAL AND IN VIVO COMPARISON WITH SQUID MEAL

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Introduction

Squid meal is widely used as a reference palatant in *Penaeus vannamei* diets, but its reliance on wild fisheries raises concerns over price volatility, supply instability, and ecological impact. Black soldier fly larvae (BSFL, *Hermetia illucens*) offer a promising alternative through their rich profile of short-chain peptides, free amino acids (fAA) and nucleotides, but their functional efficacy as shrimp palatants remains undercharacterised. This study compared the biochemical composition of a BSFL-derived ingredient with commercial palatants, and assessed if this BSFL-based ingredient could replace squid meal while maintaining or improving feed attractiveness and efficiency in *P. vannamei*.

Materials and Methods

A 17-day feeding trial was conducted at the RIASEARCH R&D facility (Portugal) using juvenile *Penaeus vannamei* (initial weight: 7.4 g; 25 shrimp/tank, n=4). Three isonitrogenous and isolipidic extruded diets were tested: a negative control (Control), a 3% squid meal diet (Squid), and a diet in which squid meal was fully replaced by 3% BSFL meal (BSF). Feed intake (%ABW/day) and FCR were calculated at trial end and analysed by one-way ANOVA with Tukey's post-hoc test ($p < 0.05$).

Selected commercial palatants, including squid meal, were sourced and submitted to physico-chemical characterisation. Nucleotide content was determined by HPLC-UV (ISO 20638:2015, Eurofins, Nantes, France), and protein solubility, molecular weight profile and fAA content (CE 152/2009) were analysed at Upscience (Saint-Nolff, France).

Results

Both the BSF and Squid diets significantly increased relative daily feed intake compared to the Control diet ($p < 0.05$), with no significant difference detected between BSF and Squid. Numerically, the feed intake of BSF diet was increased by +7.56% relatively to Control (Figure 1). The trial duration (17 days) allowed shrimps to approximately double their weight across all groups. No statistically significant differences in FCR were observed among treatments.

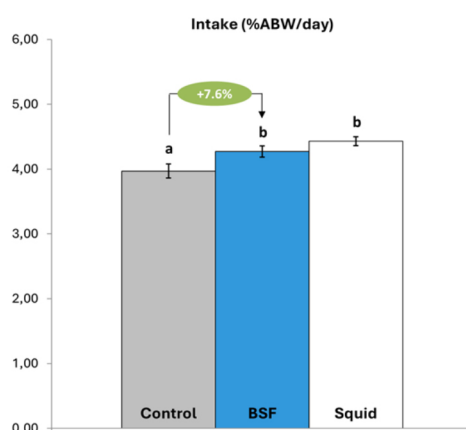


Figure 1. Average daily feed intake of *P. vannamei* fed either the control diet (Control), the BSFL-based diet (BSF), or the squid meal diet (Squid). Different letters indicate statistically significant differences between groups ($p < 0.05$).

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A separate benchmarking of Hilucia™ meal against marine-derived palatants shows that BSFL meal contains markedly higher nucleotide levels (223 mg/100g vs. 27 mg/100g for squid meal), a higher proportion of small peptides (% <1,000 Da; 58.3% vs. 34.1% for squid meal), and comparable soluble protein content (26.9% vs. 25.6% CP). Free amino acid content was also noticeably higher than that of squid meal (1.6% vs. 0.6% DM).

Discussion

These results confirm that BSFL meal included at 3% exerts a phago-stimulatory effect in *P. vannamei* comparable to squid meal, while maintaining feed efficiency. This is consistent with its biochemical composition, as nucleotides, short-chain peptides and fAA are recognised feeding stimulants in crustaceans (Suresh et al., 2011; Lee & Myer, 1997). Although the 17-day duration limits conclusions on long-term growth, it was sufficient to double animal weight and calculate FCR, and the absence of differences between BSF and Control groups suggests that increased intake would translate into faster growth at farm scale. Beyond palatability, BSFL ingredients offer meaningful advantages over marine-sourced palatants in terms of supply security, price stability and environmental footprint (Van Phi et al., 2020), complementing a growing body of evidence for beneficial effects of BSFL inclusion on shrimp feed intake, growth and disease resistance (Richardson et al., 2021; Novriadi et al., 2023; Keetanon et al., 2024). Further trials with longer durations and varying inclusion levels should be conducted to confirm dose-response relationships and validate the palatant effect of BSFL meal at commercial scale.

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BREWING BY-PRODUCTS AS REARING SUBSTRATES FOR BLACK SOLDIER FLY: EFFECTS ON LARVAL GROWTH AND CONVERSION EFFICIENCY

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Brewing by-products are recognized as suitable rearing substrates for black soldier fly (BSF), but the potential of using brewer's spent grains (BSG) and brewer's spent yeast (BSY) at different dietary ratios has rarely been investigated. This study evaluated the effects of BSG-BSY-based diets on BSF larval growth, survival, and conversion efficiency. A total of 3000 6-day-old BSF larvae were allotted to 5 dietary treatments (6 replicate boxes/diet, 100 larvae/box, 0.9g/larva): i) BSY2.5 (97.5% of BSG + 2.5% of BSY), ii) BSY5 (95% of BSG + 5% of BSY), iii) BSY7.5 (92.5% of BSG + 7.5% of BSY), iv) BSY10 (90% of BSG + 10% of BSY), and v) control (GA, Gainesville diet). Larvae were individually weighed (at least 30/replicate) every 4 days (10 and 14 days of age) and at the end of their growth (5% of prepupae), and their survival calculated. Bioconversion efficiency corrected for residue (BER), reduction rate (RR), and waste reduction index (WRI) were also calculated. Data were analysed by GLMM (IBM SPSS software, $P < 0.05$). The BSY10 larvae overall showed the highest weight among the BSG-BSY-based diets, but lower than GA ($P < 0.05$). At 10 days of age, BSY7.5 and BSY10 larvae displayed higher weight than BSY2.5 and BSY5 ($P < 0.05$), with BSY10 and GA being similar ($P > 0.05$). The 14-day-old larvae were also heavier when fed BSY7.5 and BSY10 diets than the other BSG-BSY-based diets ($P < 0.01$), but lighter than GA ($P > 0.05$). At the end of growth, the BSY10 larvae showed the highest weight among the BSG-BSY-based diets ($P < 0.05$), being analogous to GA ($P > 0.05$). Survival rates were similar among the diets ($P > 0.05$). All the BSG-BSY-based diets were characterized by higher BER than GA, with BSY7.5 and BSY10 larvae also displaying a better BER than BSY2.5 and BSY5 ($P < 0.01$). Similar RR and WRI were observed among the BSG-BSY-based diets ($P > 0.05$), but lower than GA ($P < 0.05$). In conclusion, including 7.5 and 10% of BSY in BSG-based diets led to the best BSF larval growth and conversion efficiency.

FEEDING RAINBOW TROUT WITH DIETS BASED ON *Hermetia illucens* AND *Spirulina* MEALS: IMPLICATIONS FOR GUT MICROBIOTA

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Introduction

In the last decades, the aquaculture industry has mainly directed its efforts towards the replacement of fishmeal with alternative protein sources, as a consequence of its sustainability and economic issues. Vegetable proteins have already been successfully included into commercial diets for rainbow trout, but novel feed ingredients – such as insects and microalgae – have also gained increasing attention (Sheikhzadeh et al., 2019; Biasato et al., 2022). In particular, *Hermetia illucens* (HI) meal has been reported to positively modulate the gut microbiota of rainbow trout in terms of selection of short-chain fatty acids (SCFAs)-producing bacteria and reduction of foodborne disease-causing pathogens (Biasato et al., 2022), while *Spirulina* (SP) seems to be able to promote gut microbiota diversity and increase beneficial bacteria in different fish species (Ren et al., 2022; Zhang et al., 2024). However, these two alternative protein sources have always been tested individually. Therefore, the present study aims to evaluate the impact of HI and SP meals – both individually and in different mixture percentages – on the gut microbiota of rainbow trout.

Materials and Methods

Seven isonitrogenous, isolipidic, and isoenergetic diets were formulated: a control diet (CTRL) containing 20% of fishmeal, and six diets where the fish meal was 100% replaced with HI meal (HI100), 100% SP meal (SP100), 75% HI and 25% SP (MIX1), 66.6% HI and 33.3% SP (MIX2), 33.3% HI and 66.6% SP (MIX3), and 25% HI and 75% SP (MIX4). A total of 441 fish (initial body weight of 200.5 ± 2.6 g) were randomly allotted to 21 400L-tanks (3 replicates/diet, 21 fish/tank). At the end of the trial (88 days), 6 fish per tank were slaughtered and the posterior content was collected for the characterization of the gut microbiota by means of 16S rRNA sequencing analysis. The bioinformatics analysis was performed using QIIME 2 v2.2022.2.0 (<https://docs.qiime2.org/>) importing the raw reads for quality and chimera filtering step by dada2 denoise-paired script (sampling depth 7,500). The amplicon-sequence variants (ASVs) were used for taxonomic assignment by comparison with the Greengenes2 v2022.10 databases obtaining taxa abundances. The R package microeco (v1.16.0) was used in RStudio (R v4.4.2; RStudio 2024-10-31 ucrt) to perform statistical analysis ($P < 0.05$).

Results

The 16S rDNA amplicon sequencing analysis allowed the detection of 329 bacterial genera. Observed, Fisher and Chao1 alpha diversity indices were lower in the CTRL, HI and MIX4 diets when compared to MIX3, while the latter also was also characterized by a higher Simpson and Shannon indices than the CTRL, and CTRL, HI, MIX1 and MIX4 groups, respectively (One-way ANOVA, $P < 0.05$). Inverted Simpson and Pielou indexes also resulted higher in the MIX3-fed fish in comparison with the CTRL, MIX1 and MIX4 diets (One-way ANOVA, $P < 0.05$). Differently, the beta-diversity analysis did not show a clear separation among the diets (Bray-Curtis's distances; Principal Coordinates Analysis [PCoA], $P > 0.05$). However, Permutational Multivariate Analysis of Variance (PERMANOVA) demonstrated differences in gut microbiota composition between the CTRL and HI100 diet ($F = 4.06$, $R^2 = 0.30$, $P < 0.05$). Part of the taxa resulted differentially abundant among the groups, with *Weissella* spp. resulting less abundant in the CTRL and SP100 groups when compared to HI100-fed fish, and *Streptococcus* spp. being lower in the SP100 diet than the HI100 group (Dunn's Test of Multiple Comparisons, $P_{adj} < 0.05$). Lastly, each dietary treatment displayed a distinct set of unique bacterial genera, thus indicating a strong selective pressure on the gut microbiota of the fish. In particular, the SP100 diet showed the highest number of diet-exclusive taxa (including *Holdemanella*, *Paraeggerthella* and *Gallalistipes*), followed by MIX3 and MIX4 groups (mainly characterized by *Cellulomonas*, *Trichococcus* and *Nocardioides*).

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Discussion

The results herein obtained suggest that the diets containing the highest percentages of inclusion of SP meal (SP100, MIX3 and MIX4) were able to exert a most pronounced effect on the gut microbiota of the rainbow trout, as a consequence of its highly degradable polysaccharides and fermentable oligosaccharides (which led to the selection of unique bacterial genera associated with carbohydrate degradation and fiber fermentation) (ibrahim et al., 2026). However, the highest alpha diversity overall identified for the MIX3 diet interestingly suggests a potential synergy between SP and HI. Indeed, the concomitant presence of chitin (cellulose-like, insoluble fiber) – which reasonably explains the selection of cellulose-degrading (*Weissella*) (Biasato et al., 2022) and degraded chitin-using (*Streptococcus*) (Yildirim-Aksoy et al., 2019) ASVs in the HI100 group – and fermentable oligosaccharides (soluble fibers) may offer more diversified nutrients for the gut microbiota.

Acknowledgment

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OYSTER STOCKING DENSITY MODULATES PRODUCTIVE OUTPUT IN A LAND-BASED MULTI-TROPHIC SYSTEM

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Introduction

Recirculating multi-trophic aquaponic systems integrate fed aquatic species with organic and inorganic extractive organisms to reuse water and convert residual outputs into additional products (Granada et al., 2016; Spradlin & Saha, 2022). However, saltwater applications remain underdeveloped, and the biomass ratio among trophic compartments requires optimization. Within the SuInAp project, this study evaluated whether Pacific oyster (*Crassostrea gigas*) stocking density affected water quality, gilthead seabream (*Sparus aurata*) performance and product quality, and feed-normalized output in a system also producing edible salicornia.

Materials and Methods

Nine independent systems were allocated to 0, 30 or 60 oysters per system (F-O0, F-O30 and F-O60; three replicate systems per treatment) for 134 d. Each system included a fish tank, a 5-L settling tube, an oyster tank, a biofilter, cartridge filtration and ultraviolet treatment, plus an independent four-level nutrient film technique unit for salicornia. Twice weekly, 100 L were transferred from the biofilter to the plant reservoir and 100 L from the plant reservoir to the oyster tank. Water quality was monitored throughout the trial. Fish growth, feed conversion and survival were evaluated from day 0 to 134. Four fish per system were sampled for somatic indices, processing yields, muscle pH and scale cortisol (ng g⁻¹ matrix). Apparent system biomass productivity was calculated as fish biomass gain plus whole-oyster biomass gain plus harvested edible salicornia biomass per unit feed; extractive productivity included oyster gain and salicornia only. The system was the experimental unit. Preliminary treatment comparisons used exact randomization tests on system-level values and time-weighted water means.

Results

Time-weighted temperature, dissolved oxygen, pH, turbidity, chlorophyll, ammonium, nitrate and phosphate did not differ among treatments. Initial fish weight averaged 78.3-81.2 g and final weight 153.1-169.5 g. Specific growth rate (0.497-0.548% d⁻¹), feed conversion ratio (1.41-1.47) and survival (86.7-93.3%) were unaffected by oyster density. Apparent system biomass productivity was 2.60 ± 0.62, 3.86 ± 0.70 and 2.65 ± 0.46 kg fresh product kg⁻¹ feed in F-O0, F-O30 and F-O60, respectively (P = 0.079). Extractive productivity was highest in F-O30 (1.90 ± 0.48, 3.13 ± 0.69 and 1.94 ± 0.28 kg fresh extractive biomass kg⁻¹ feed; P = 0.046). Oyster density did not affect viscerosomatic or hepatosomatic indices, carcass yield (93.7-94.3%), fillet yield (46.4-47.1%), edible meat yield (37.0-38.5%), muscle pH or scale cortisol (1080-1350 ng g⁻¹ scales).

Discussion

The intermediate oyster density increased feed-normalized extractive output by approximately 65% relative to F-O0 and 61% relative to F-O60, without detectable impairment of water quality, fish growth, feed utilization, processing yields or scale cortisol. The non-linear response indicates that increasing the number of extractive organisms does not necessarily maximize whole-system production, emphasizing the need to balance trophic biomass and system design (Nederlof et al., 2022). These preliminary findings support the concept that integrated production of fed and extractive species can diversify fresh-food output while maintaining seabream performance and physiological status. Direct nutrient-retention measurements will be required to distinguish productive output from nutrient-use efficiency.

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RESULTS FROM THE SMART TANG PROJECT: SCALING NET-BASED CULTIVATION OF SUGAR KELP (*Saccharina latissima*) IN THE LIMFJORDEN, DENMARK

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The SMARTTANG project (2022–2025) aimed to develop, test, and document scalable cultivation strategies for sugar kelp (*Saccharina latissima*) using tube and net-based systems adapted from existing nearshore mussel aquaculture infrastructure.

Through systematic hatchery trials, testing different seeding techniques and net materials (nylon, polyester, polypropylene) a final seeding was made, seeding five 50 m nets with a depth of 1.2 m with *S. latissima*. The nets were deployed in a commercial mussel farm in Limfjorden, Denmark. The field trials documented, that the biomass yields were controlled by depth and vertical/horizontal deployment orientation: Nets deployed horizontally near the surface achieved the highest yields, with up to 13.2 ± 2.4 kg fresh weight m^{-2} , compared to 7.2 ± 0.7 - 8.4 ± 0.3 kg m^{-2} on vertically oriented nets and 4.9 ± 0.5 kg m^{-1} on a horizontal line. The biomass quality was suitable for food, feed, and biorefining applications, though iodine concentrations and biofouling increased markedly toward harvest in late April, underscoring the need for early harvest.

Trials were conducted for harvesting kelp of the nets testing existing mussel harvesting equipment, but it was apparent that technology development was needed to effectively harvest the nets. The harvested biomass was processed at a large scale facility using a screw press and a microcutter to obtain a biomass slurry, which was readily fermentable with addition of a lactic acid bacteria inoculum. The fermented biomass stored well, and was still useable and pH stable after 1 years of storage, after which it was tested in a commercial scale setting as feed for pigs. The raw biomass was also used for development of a new snack for kids, which was evaluated within the project.

Finally, a complementary sustainability assessment integrating Life Cycle Assessment and Techno-Economic Analysis compared net-based systems with conventional longline cultivation. While net systems achieved high area-specific yields, longline systems showed substantially lower material intensity, greenhouse gas emissions and higher economic performance.

Overall, the SMART TANG project demonstrated that *S. latissima*, can be produced on tube-net systems, and used for food and feed, but longline systems currently remain superior in terms of environmental footprint and economic viability. The results provide concrete guidance for seaweed producers, technology developers, and policymakers seeking to scale-up nearshore kelp cultivation in European waters.

ASSESSMENT OF BIOACTIVE PEPTIDE ENRICHED DIETS ON GROWTH AND ROBUSTNESS OF EUROPEAN SEABASS (*Dicentrarchus labrax*) POST-LARVAE

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Introduction

Fish in aquaculture are frequently exposed to inherent stressors that can impair growth performance and survival. Nutritional strategies based on the use of functional ingredients have appeared as a promising approach to enhance fish robustness and improve resilience to stress. Bioactive peptides obtained from alternative protein sources have shown potential to enhance growth, modulate immune and antioxidant responses, and improve stress resilience in fish. The present study evaluated the effects of diets supplemented with alternative peptide fractions on the growth performance, stress response, antioxidant status, and immune function of European seabass (*Dicentrarchus labrax*) post-larvae.

Materials and Methods

Five experimental diets were evaluated: a commercial control diet (D1), two poultry-based diets in which fish-derived protein ingredients were partially replaced by chicken protein hydrolysate and poultry meal (D2) or feather meal hydrolysate and poultry meal (D3), a yeast-based diet (D4), and a diet containing wheat hydrolysate and whey protein isolate (D5). European seabass post-larvae were reared under controlled conditions in a closed system from 84 to 112 days after hatching (DAH). Fish were assessed for growth performance, antioxidant and immune responses, and stress resilience before and after an acute stress challenge, consisting of confinement of fish at the bottom of the tanks for 30 min. Analysed humoral and oxidative stress parameters, from digestive tract tissue, included lysozyme activity, nitric oxide levels, peroxidase activity, bactericidal activity, catalase activity, superoxide dismutase (SOD) activity, glutathione peroxidase activity, and thiobarbituric acid reactive substances (TBARS). In addition, the expression of molecular biomarkers associated with stress, antioxidant defence, and immune regulation (*hsp90*, *hsp70*, *gr1*, *gr2*, *il10*, and *sod1*) was analysed.

Results and Discussion

An overall analysis of the growth indicators reveals that, in this case, the performance of diets D2 and D3 were similar to the commercial control (D1). Indicating that a partial replacement of the traditional fish protein hydrolysate and fish meal by chicken protein hydrolysate and poultry meal (D2), and by feather meal hydrolysate and poultry meal (D3), did not impact, positively or negatively, the growth performance of European seabass post-larvae. However, fish fed the diet supplemented with wheat hydrolysate and whey protein isolate (D5) showed the lowest weight, length and relative growth rate. Survival rates were statistically similar throughout all the diets at the end of the trial (112 DAH).

Regarding antioxidant and humoral responses, the D2 diet significantly increased *sod1* gene expression and nitric oxide levels at the end of the feeding period, while significantly reducing peroxidase activity immediately following the stress challenge. No significant differences in bactericidal activity were observed among groups prior to the stress challenge. D1 fed fish showed an increase in bactericidal activity following the challenge, whereas fish fed D3 presented a reduction. Most supplemented groups showed a similar reduction trend, although their values remained at intermediate levels and did not differ significantly from those of either D1 or D3. Lysozyme activity remained stable before and after stress, but an increase was observed during the recovery period, in D5 fed fish. These molecular responses were not accompanied by changes in oxidative damage biomarkers, indicating that the effect was not sufficient to alter the oxidative status of the fish under the conditions tested. This discrepancy is consistent with the relatively low mortality of the stress challenge, which likely did not produce the physiological impact necessary.

Results suggest a dietary induced modulation of redox-responsive pathways, that may have resulted in an improved antioxidant preparedness prior to stress exposure, in fish fed the chicken protein hydrolysate and poultry meal diet (D2). In addition, the experimental treatments influenced innate immune responses to the stress challenge, particularly in fish fed D3 (feather meal hydrolysate and poultry meal diet), by affecting the activation of immune defence mechanisms.

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Overall, the present study demonstrated that alternative peptide fractions can influence the robustness and resistance of European seabass post-larvae, while maintaining a growth performance comparable to that of a commercial-like diet. The most promising responses were observed in fish fed a diet containing chicken protein hydrolysate and poultry meal. However, further studies using a more suitable stress challenge are needed to confirm these effects and validate their biological relevance.

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TOXICOLOGICAL RESPONSES OF BIVALVE SPECIES (*Mytilus edulis* AND *Ruditapes philippinarum*) UPON CHRONIC EXPOSURE TO PETROLEUM- AND BIO-MICROPLASTIC PARTICLES

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Introduction

Bivalve aquaculture plays a key role in global seafood production, with many species reared in coastal areas that are highly exposed to anthropogenic contamination, including marine litter (Van Der Schatte Olivier et al., 2020). As sessile and filter-feeding organisms, bivalves are particularly prone to accumulate contaminants and suspended particles, e.g. microplastics, which may pose risks from ecotoxicological and seafood safety standpoints (Tanaviyutpakdee & Karnpanit, 2023). To counteract this pervasive problem, the plastic industry has invested in the development of “eco-friendly” alternative materials made from natural and renewable sources and/or with the capacity to biodegrade (Rosenboom et al., 2022). However, and, in case of bio-degradable polymers this tendency is higher than for conventional plastics, as bio-fragmentation occurs simultaneously to typical fragmentation from natural drivers (UV-radiation, temperature, oxygen and mechanical stress). In addition, literature regarding the ecotoxicological aspects of bio-based plastics is scarce, thus, failing to support the sustainability and “eco-friendliness” claims these materials hold. Hence, this study aimed to investigate the ecotoxicological responses of two bivalve species (*Mytilus edulis* and *Ruditapes philippinarum*) extensively farmed in Europe, upon exposure to microplastics derived from commercially relevant petroleum-based (polyethylene terephthalate, PET), partially bio-based (polybutylene succinate, PBS) and bio-based plastics (poly[propylene] 2,5-furandicarboxylate, PPF).

Materials and Methods

M. edulis (~ 20g) and *R. philippinarum* (~13 g) were kept in glass beakers with ~5 L seawater under optimal rearing conditions (18.0 ± 0.5 °C, 8.0 ± 0.2 pH, > 7 mg/L O₂). During 21 days of trial, bivalves were daily fed with a *Tetraselmis* spp. solution once a day and exposed via water to 1 mg/mL of three types of microplastics: i) PET; ii) PBS; or iii) PPF. Bivalves were sampled from each treatment after 72 hours (T₇₂) and 14 days (T₁₄) of microplastic exposure and after 7 days (day 21, T₂₁) of depuration period. The digestive glands and gills were collected to evaluate different physiological endpoints: oxidative stress (catalase activity [CAT], superoxide dismutase [SOD], glutathione S-transferases [GST], and lipid peroxidation [LPO]), metabolic enzymes activity (lactate dehydrogenase [LDH] and citrate synthase [CS]), digestive enzymes activity (α -amylase, pepsin, and trypsin), and animal condition indexes.

Results and Discussion

In mussels digestive gland, significant differences were found in CAT (T₇₂), SOD (T₇₂, T₁₄, and T₂₁), and GST (T₇₂, T₁₄, and T₂₁). CAT was upregulated by the exposure to PBS (T₇₂). SOD was upregulated by the exposure to both PPF and PBS at T₇₂ and T₁₄, and downregulated after 7 days of the depuration period (T₂₁) in PET treatment. GST was upregulated by PET in both T₇₂ and T₂₁, while a downregulation was observed in PBS at both T₇₂ and T₂₁, and in PPF at T₂₁. These patterns suggest that the antioxidant defense system of the mussels gland was differently activated depending on the polymer type and trial phase. The upregulation observed at T₇₂ indicated an early response to oxidative stress in mussels exposed to PBS and PPF exposure. However, the persistence of altered SOD and GST activities at T₁₄, and the downregulation observed after the depuration period (T₂₁), may reflect a prolonged physiological effect or an inability of the antioxidant system to fully recover even after 7 days of ceasing microplastics exposure.

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These preliminary results highlight that the exposure to bio-microplastics may not be as environmentally harmless as it is often claimed. Despite being produced in more sustainable ways, alternative polymers such as PBS and PPF, may induce oxidative stress and disrupt key physiological processes in wild and/or extensively farmed marine species. This raises concerns for the aquaculture sector, because if a shift towards more sustainable and supposedly eco-friendlier alternatives occur, it is important to ensure that these materials will not negatively impact animal health, productivity, and ultimately result in microplastic accumulation in the edible fraction of seafood. Therefore, ensuring that novel materials are not only bio-derived but fully biodegradable, as well as non-toxic to marine organisms is important to assure a sustainable aquaculture practices and seafood safety.

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PHYSIOLOGICAL RESPONSES OF EUROPEAN SEABASS (*Dicentrarchus labrax*) TO CHRONIC VESSEL NOISE IN OFFSHORE CAGE FARMING

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Introduction

Anthropogenic underwater noise is an increasing stressor in marine environments, largely driven by maritime activities. Vessel traffic represents the dominant source of low-frequency noise in coastal areas, overlapping with the hearing range of many fish species, including European seabass (*Dicentrarchus labrax*) (Popper and Hawkins, 2019). As seabass rely on acoustic cues for communication and environmental perception, vessel noise may disrupt these processes and induce physiological stress. Previous studies have shown that boat noise can activate the stress response in fish, increasing cortisol levels and altering metabolic activity (Širović et al., 2026). While these effects may be adaptive in the short term, repeated exposure could impair welfare and performance. This is particularly relevant in aquaculture, where seabass is often reared in coastal areas with frequent vessel traffic. However, the long-term effects of noise exposure under real farming conditions remain poorly understood. This study aims to investigate the physiological responses of European seabass to chronic vessel noise in offshore sea cage systems.

Materials and methods

Adult European seabass (n=144; mean weight±SD: 336.3±78.6g) were chronically exposed to daily 1-hour playbacks of sudden unfamiliar boat noise in three offshore sea cages at the fish farm Valle Ca' Zuliani srl (Monfalcone, Italy). Sampling was conducted at predefined time points to assess physiological responses over time. A representative number of fish (n = 12 per cage at each sampling point; three cages in total) were collected after the first sound exposure (T1), after 1 month of daily exposure (T2), and after 2 months of daily exposure (T3). A baseline sampling (control, T0) was performed 30 days prior to the onset of experimental noise exposure. Fish sampling was carried out after the sound exposure and, immediately after capture, fish were euthanized and blood and scales collected. Cortisol concentrations were quantified by radioimmunoassay (RIA) in plasma and scales to assess the primary stress response (Meloni et al., 2025). Secondary stress indicators, including plasma glucose, lactate, and heat shock protein 70 (HSP70) levels, were measured using an automated analyser (BIONSEN, EKF Diagnostics) and a commercial ELISA kit (Cusabio), respectively. Total proteins (TP) as additional stress indicator and Advanced Oxidation Protein Products (AOPP) as oxidative stress marker were assessed using a BCA kit (ThermoFisher) and the protocol outlined by Witko-Sarsat et al. (1998), respectively. Blood smears were prepared from heparinized entire blood and the differential leucocyte was performed to check for the fish health. Data were analyzed using a linear mixed model with condition and sex as fixed factors, weight as covariate and tank as random factor.

Results and discussion

Chronic vessel noise influenced physiological responses in European seabass, with different results according to the explored stress indicators. Plasma cortisol increased at all exposure times compared to the baseline period (T0), with the highest levels at T3 (after two months of exposure) (147 ng/ml; p<0.001). In contrast, scale cortisol showed a different pattern, with a significant increase only at T2 (after 1 month of exposure) (0.92 ng/g vs. 0.14 ng/g; p<0.05) and remained stable up to T3. Plasma glucose decreased at T2 compared to T0 (210 mg/dL vs. 103 mg/dL; p<0.05), followed by a marked increase from T2 to T3 (103 mg/dL vs. 224 mg/dL; p<0.05), which may indicate a shift in energy allocation along exposure. Lactate increased significantly from T0 to T2 (9.09 mmol/L vs. 13.7 mmol/L; p<0.05), suggesting potential elevated metabolic demand during exposure. Cellular stress response (HSP70) showed a significant increase only at T2, while the AOPP/TP ratio increased after the first exposure. Neutrophil-to-lymphocyte ratio (NLR), an indicator of chronic stress, was not significantly affected (0.11). Overall, these results indicate that chronic vessel noise exposure may influence endocrine and metabolic homeostasis. However, no clear evidence was found to indicate a chronic stress.

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Conclusions

The data indicate an initial physiological stress response, reflected by early changes in oxidative stress markers, immune parameters, glucose, and lactate, which partially attenuate over time. Low scale cortisol levels in the 2-month exposure groups, along with the absence of an elevated neutrophil-to-leukocyte ratio, do not support the development of chronic stress. Some variations in acute indicators (plasma cortisol, glucose, and lactate) may instead reflect transient disturbances from cage farming and handling rather than noise exposure per se. Overall, chronic vessel noise, under the tested conditions, induces measurable early physiological responses in farmed European sea bass without clear evidence of chronic stress. Future studies are needed to investigate whether different types of vessel noise, as well as varying modes and timing of exposure, may produce different effects in fish.

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LIGNOCELLULOSE IN DIETS OF RAINBOW TROUT *Oncorhynchus mykiss* ON FAECAL INTEGRITY AND WATER QUALITY PARAMETERS IN RECIRCULATING AQUACULTURE SYSTEMS

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Introduction

In recirculating aquaculture systems (RAS), water quality depends on the continuous removal and transformation of feed residues and fish waste. System stability is highly sensitive to waste accumulation, making effective mechanical and biological filtration essential to control suspended solids, nitrogen compounds, and chemical oxygen demand. Faecal integrity strongly affects removal efficiency: well-formed faeces are easily captured, whereas fragmented faeces break into fine particles that are harder to remove. These increase turbidity and organic load, leading to higher oxygen demand, reduced biofilter performance, and overall deterioration of water quality. This may lead to a decrease in fish performance.

Lignocellulose, derived from soft wood, is known to take positive influence on faecal integrity in companion animals and livestock. This study aims to investigate the effect of lignocellulose on fish performance, faecal integrity and water quality parameters in rainbow trout (*Oncorhynchus mykiss*) reared in RAS.

Materials and Methods

Rainbow trout with an average weight of 57 g were used for this trial. The animals were acclimatized to rearing conditions (2 weeks) and subsequently homogeneously allocated (240 - 250 animals) to one of 12 identical Ecotrons (RAS units) with 2.000 L tanks. Each group was fed with one of 3 iso-nitrogenous, iso-lipidic and iso-energetic diets containing graded inclusion levels of lignocellulose at 0, 1.0 and 2.0 %. In total 2920 animals were initially stocked and four ecotron replicates were used per diet.

After six weeks zootechnical performance data and faeces (striping 6 fish per unit) were collected. Water oxygen concentration and pH were continuously monitored and standard quality parameters (NO₂- and NH₄+) including chemical oxygen demand (with small scale tube method; ST-COD) were measured once a week. The faecal score was conducted using a 3-level scoring scale with observational faecal consistency: 1: no structure (diarrhea), 2: some structure/firmness and 3: solid faecal structure.

Results

The inclusion of lignocellulose in the diets of rainbow trout did not significantly influence zootechnical parameters. The evaluation of faecal integrity and water quality showed significant influence of lignocellulose compared to the control group (see table).

Table

Results of ST-COD and Faecal Scoring

Group / Parameter	ST-COD average (mg O ₂ /L)	SD	Faecal Score average	SD
Control	12.2 ^a	1.72	1.9 ^a	0.39
1 % Lignocellulose	8.0 ^b	2.96	2.8 ^b	0.08
2 % Lignocellulose	6.1 ^b	0.89	2.6 ^{ab}	0.25

a,b: Different superscripts in a column indicate significant differences. COD: (p<0.01); Faecal Score (p<0.05); 1: no structure (diarrhea), 2: some structure/firmness, 3: solid faecal structure. SD: Standard deviation.

(Continued on next page)

Discussion

Lignocellulose, an insoluble dietary fiber composed of cellulose, hemicellulose, and lignin, plays an important role to manage faecal consistency in companion animals i.e. dogs (Abd El-Wahab et al. 2022) and livestock animals i.e. broiler chicken (Farran et al. 2013). Lignocellulose supports gut functionality by stimulating peristalsis and improving intestinal transit. Due to its high water binding capacity, it absorbs and retains free water in the gut, increasing digesta bulk and promoting the formation of firmer, more cohesive faeces.

This feeding trial in rainbow trout (*Oncorhynchus mykiss*) with inclusion of 1 % and 2 % lignocellulose in the diet compared to a control group shows the efficiency to promote faecal integrity in a new species and environment. In RAS faecal consistency strongly affects waste removal efficiency. The stability of the system is highly dependent on effective removal of faeces from the fish tanks and culture water to control water quality. The significant improvement of the faecal score in this trial between control and 1% lignocellulose inclusion is accompanied by the decrease of ST-COD, a water quality parameter, for lignocellulose enriched diets up to 2%. ST-COD is considered a parameter directly linked to waste removal efficiency.

Further investigations are needed (other fish species, other breeding systems, other sources of lignocellulose) to understand the mode of action and confirm the interest of dietary lignocellulose supplementation in aquaculture.

Acknowledgment

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EXPLORING FINANCIAL VIABILITY OF CIRCULAR BIOECONOMY INNOVATIONS IN EUROPEAN FRESHWATER AQUACULTURE

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Introduction and objectives

It is a long-standing critique of private enterprise that management is incentivized to optimize financial return to shareholders over other objectives such as resource use efficiency or avoidance of environmental and social harms. This has been countered through many initiatives including the World Economic Forum Stakeholder Capitalism initiative (World Economic Forum, 2020) and the EU Green Deal strategy (European Commission (2019)). The concept of the circular economy has emerged as a practical strategy to improve sustainability whilst supporting continued economic growth (Tuladhar, A. et. al., 2022). The SmartAqua4FuturE (SAFE) project is one of many that is exploring technical innovations that support a more circular bioeconomy especially in the context of European freshwater aquaculture. As one component of the project, the authors examined the financial viability of candidate technologies from a business perspective to help identify opportunities and constraints that might be relevant for sector stakeholders and governance organisations.

Approach and methods

The main approach taken was that of a marginal analysis, calculating the additional costs of implementing a technology and comparing it with the additional income that could be generated, or savings that might be made through avoiding previous costs. Spreadsheet models were used to conduct a sensitivity analysis to help identify the most critical elements contributing to net benefits and determine potential financial incentives for adoption. The main challenge was access to industry cost data, making it necessary to use a mixture of actual data, information from academic literature and estimated costs from other financial models. The other main issue to consider was the likely impact of operational scale on costs and returns. Data generated within the project was at experimental or pilot scale and potentially unrepresentative of costs and returns at commercial scale.

Results

The main example highlighted concerns the collection of sediment from carp ponds in Poland using straw bales which can subsequently be incorporated into substrate for oyster mushroom cultivation. Oyster stem byproducts can then be incorporated into carp diets, especially those used to prepare juvenile fish for over-wintering. Experimental work showed this to be technically feasible and economically interesting in terms of scale as Poland is both a major producer of farmed carp and oyster mushrooms. Financial analysis suggested that even as a byproduct, oyster mushroom stems would be an expensive feed ingredient, but at low inclusions rates it acts as a functional feed and improves over-wintering survival rates sufficiently to justify the additional costs involved. The collection of pond sediment for incorporation in mushroom substrate appears uncompetitive with current alternatives. However, a second example provides an interesting counterpoint. Analysis of the marginal costs of using a high-efficiency drier to produce biofertilizer from recirculated salmon system sludge in Norway appeared financially attractive. However, the sensitivity analysis showed the most important factors to be the high cost of electricity in the test location and the disposal fees avoided by treating and selling rather than dumping the waste sediment. Where electricity prices are lower, a less efficient drier at lower capital cost would be more cost effective, and where sediment disposal fees are low or non-existent, biofertilizer production is uneconomic. This is broadly the case in Poland where there is no cost for releasing suspended solids from pond aquaculture where carp production is below 1.5 t/ha/yr. If an appropriate solids discharge fee were introduced in Poland, the collection and utilisation of pond sludge would become more financially attractive as a means of reducing total costs rather than increasing farm income.

Discussion

The results suggest that there will be some circular economy innovations that provide financial advantage and are likely to be adopted by aquaculture enterprises providing the technology is robust and there are no significant regulatory barriers. Other innovations are unlikely to be adopted if they impose additional cost on the producer unless they become essential due to regulatory or market drivers. Encouraging enterprises to increasingly internalize their externalities almost inevitably leads to higher prices for consumers, but could lead to lower costs for society as a whole. There is therefore a need for both technical and policy innovation to support the emergence of more circular businesses.

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Acknowledgment

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EFFECTS OF INCREASED DIETARY PHOSPHOLIPIDS ON GILTHEAD SEABREAM (*Sparus aurata*) REARING FROM JUVENILE UP TO COMMERCIAL SIZE

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Introduction

It has already been established through research that fish larvae have high nutritional needs in phospholipids. Phospholipids (PLs) have a prominent role as structural components of cellular membranes, and at the larval stage the formation of tissues and organs is still underway (Sargent et al. 2002; Saleh et al. 2012). At the same time, not much is known about the PL needs of fish in the juvenile stage or later. This may be because there is some evidence, albeit limited, of the existence of a PL biosynthesis mechanism in fish (Sargent et al. 2002). Currently in Mediterranean aquaculture improved growth rates in fish have been achieved through the application of certain genetic selection programs (Ferosekhan et al. 2022). Knowing that faster weight gain also means faster tissue development, questions are raised about whether the needs of fish in structural nutrients, such as PL, are covered only by their biosynthetic ability. 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 (*Sparus aurata*) of commercial size. Simultaneously, the efficiency of PL sources of marine and plant origin is explored.

Methodology

Five different diets were formulated and produced, of which one was used as a Control diet (typical composition of commercial diets, PL 0.45%). The rest of the diets contained, in pairs, krill oil (K) and soybean oil (S) in two levels (PL 0.7% and PL 1.0%). This was to achieve a 50% and 100% increase in PL content compared to the Control diet. Going forward the diets will be referenced as K50, K100, S50 and S100. Fifteen homogeneous groups of 10 juvenile gilthead seabream with initial body weight (BW) 28.0 ± 0.13 g (triplicated groups for each treatment) were used. Fish were fed according to water temperature and BW. Mean water temperature was 21.1 ± 0.2 °C. When the fish reached commercial size, the trial ended (total duration: 8 ½ months), weight and length were measured and blood samples, as well as liver, intestine and fillet tissues were taken. The growth parameters that were investigated include: fish weight (W) and its coefficient of variation (CV_w), specific growth rate (SGR), feed conversion ratio (FCR), thermal growth coefficient (TGC) and organosomatic indices. Additionally, the physiological condition of the fish was evaluated by analyzing blood samples (cholesterol, glucose, albumin, triglycerides, total protein, total antioxidant capacity, catalase). Sample analysis is still ongoing, so presented below are the results that have been gathered up until now.

Results

Fish weight was significantly lower in the Control treatment. The K100 diet resulted in higher final weight, followed by K50. The S50 and S100 diets showed no significant differences from the Control diet. Similar results were obtained for SGR and TGC. CV_w and FCR did not show significant differences among treatments. Serum biochemical parameters weren't significantly different from control except for cholesterol, which was higher in fish that consumed K100. Organosomatic indices (Hepatosomatic index, spleenosomatic index, viscerosomatic index, perivisceral fat somatic index) didn't show significant differences among treatments.

Table 1: Results of selected parameters analyzed for the experiment.

	Control	K50	K100	S50	S100	P
Weight (final), g	326.1±9.3 ^a	350.5±2.0 ^b	371.4±2.7 ^c	343.6±3.9 ^{ab}	337.8±5.8 ^{ab}	**
CV_w , %	10.2±1.8	9.7±2.6	9.3±1.3	7.8±3.7	9.6±1.3	ns
SGR	0.943±0.01 ^a	0.972±0.0005 ^b	0.995±0.003 ^c	0.964±0.004 ^b	0.967±0.008 ^{ab}	**
FCR	1.35±0.01	1.34±0.02	1.31±0.01	1.37±0.02	1.36±0.01	ns
TGC	0.705±0.011 ^a	0.736±0.0005 ^b	0.761±0.003 ^c	0.727±0.004 ^b	0.720±0.009 ^{ab}	**
FR, %	1.069±0.003	1.072±0.015	1.061±0.010	1.091±0.013	1.083±0.002	ns
Cholesterol, mg/dl	336.2±39.0 ^{ab}	372.9±2.1 ^{abc}	422.8±20.9 ^c	328.7±13.1 ^a	403.9±9.9 ^{bc}	*

P: Significance Level; TAC: Total Antioxidant Capacity; HSI: Hepatosomatic Index ns: not significant, P>0.05; * P<0.05; ** P<0.01

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Discussion

Present results suggest that the genetically improved gilthead seabream that are currently used in intensive aquaculture have higher PL needs. Also, there is an indication that krill oil is a more efficient source of PLs than soybean oil. At the same time, the physiological state of the fish is not affected, as indicated by the analysis of the samples. To our knowledge, there is a limited number of studies regarding PL needs in Mediterranean aquaculture fish species, while there is little to no research on adult fish. Despite this, Kokou et al. (2021) reported similar results for gilthead seabream rearing from 12 to 50 g. Conclusions regarding growth, in addition to the physiological condition of the fish, will be clearer after further analysis of the samples. Nonetheless, there seems to be potential in increasing the phospholipid level of gilthead seabream growout diets.

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EMBRYONIC THERMAL HISTORY AND ITS PERSISTENT EFFECTS ON CARDIAC MOLECULAR RESPONSES IN ATLANTIC SALMON

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Introduction

In Atlantic salmon aquaculture, the application of specific thermal regimes, particularly the use of higher temperatures to accelerate embryonic development, is a widely used strategy to optimise production efficiency. However, accumulating evidence indicates that promoting rapid growth through higher temperatures during early development may entail substantial physiological costs, with potential long-term consequences for fish health, robustness, and the structural integrity of vital organs. Recent evidence further suggests that lower embryonic temperatures may represent a more favourable approach, conferring lasting beneficial effects on the fish. The heart, which exhibits pronounced plasticity during early ontogeny, is especially susceptible to thermally induced developmental perturbations. In this study, we investigate how different embryonic temperature conditions influence cardiac molecular responses to challenge, both *in vitro* and *in vivo*, in Atlantic salmon, with a particular focus on genes involved in cellular stress responses and tissue structural organisation.

Materials and Methods

Fertilised eggs were exposed to three distinct thermal regimes from fertilisation until the eyed stage. These included a constant-temperature group maintained at 8 °C, a cold-exposure group maintained at 4 °C, and a fluctuating-temperature group following an 8, 4, and 8 °C profile. After this period, all groups were transferred to a standardised temperature of 8 °C for the remainder of embryonic development and hatching, and were maintained at this temperature until the onset of exogenous feeding. Fish were subsequently reared at 12 °C until the end of the trial. Cardiomyocytes were isolated at the parr and smolt stages and subjected to experimental stressors, including oxidative stress and induced hypoxia. Cellular responses were assessed 24 hours after exposure. For the *in vivo* stress trial, Atlantic salmon smolts were subjected to a stress challenge involving 10 seconds of air exposure followed by 30 minutes of confinement. Heart samples were collected before stress, immediately after stress, and at 4 and 8 hours post-stress. From each heart, the ventricle, atrium, and bulbus arteriosus were dissected and analysed separately for gene expression.

Results

Preliminary data reveal pronounced heterogeneity in gene expression across cardiac tissues and embryonic thermal regimes. In the *in vitro* challenge experiments, cardiomyocytes exhibited distinct expression profiles linked to their thermal history. Exposure to hypoxia resulted in significant interaction effects between embryonic temperature and treatment, characterised by a strong induction of heat shock protein genes alongside a concurrent downregulation of the marker *cyp11a1*. Similarly, oxidative stress elicited significant interaction effects in several key biomarkers, with the induction of *hsp70* and antioxidant defence enzymes, including *gstt*, *mnsod*, and *gpx*, being clearly modulated by embryonic temperature. While expression levels of these markers remained stable or declined in the constant 8 °C group, they showed modest upregulation in the 4 °C and 8, 4, and 8 °C groups. Samples from the *in vivo* stress trial are currently being processed, and the results will be presented at the conference.

Conclusion

Embryonic thermal history clearly shapes cardiac molecular responses to later life stress in Atlantic salmon, with lower or fluctuating temperatures conferring distinct regulatory effects on key stress and antioxidant pathways. The differential induction of heat shock proteins and antioxidant defence genes suggests that cooler embryonic conditions may promote enhanced cardiac stress resilience. These findings highlight the heart as a sensitive target of early thermal programming and underscore the need to balance short term production gains with long term physiological robustness in aquaculture. Ongoing *in vivo* analyses will further clarify the functional relevance of these molecular patterns at the whole organism level.

Acknowledgment

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A COLLECTIVE INITIATIVE TO ASSESS DOMESTICATION POTENTIAL AND GENETIC IMPROVEMENT OF ALGAE SPECIES IN FRENCH AQUACULTURE

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Introduction

French algaculture is diverse but remains limited in terms of production, with 283 T of macroalgae, 430 T of spirulina and 9.3 T of microalgae produced in 2024 (Agreste, 2024). To date, the management of genetic resources and the implementation of genetic improvement practices in algae are poorly documented. In micro-algae and spirulina, genetic selection is limited by current cultivation practices, which rely on asexual reproduction. In macro-algae, diverse cultivation practices have been described, either related to sexual reproduction of introduced *Undaria pinnatifida* (Guzinski et al., 2018) or to the use of wild, locally-collected strains to produce F1 progeny, generally without any selective breeding approaches (Goecke et al., 2020). International guidelines recommend that genetic improvement of macro-algae follows the principles established for terrestrial crops, including the development of homogenous, distinct and stable varieties or cultivars as certified commercial products (FAO, 2019). The arguments for adopting such breeding strategies are however questionable, regarding the size and the stage of development of the French algal production.

Advances in international research have reported proof of concept studies aimed at developing new selective breeding practices in micro- and macro-algae, similar to those used in terrestrial and aquaculture species in populations with large genetic variability and established pedigree-based selective breeding practices (Bråtelund et al., 2026; Mauger et al., 2026; Nehr et al., 2025). Successful industrial implementation of such innovations will require 1) improved characterisation of current genetic practices, socio-economical and technical contexts and 2) establishment of selective breeding models and the necessary technical and administrative ecosystem, including regulations.

To capitalize on these opportunities, a French national and collective initiative was launched in 2026 through the TEHASIS project. The project aims to bring together algal producers, scientific and technical stakeholders, including Ultra-Peripheral Regions (UPR) and Overseas Countries and Territories (OCT), with the objective of fostering innovation transfer and establishing the foundations for future algal breeding programs.

Objectives

TEHASIS (2026-2028) aims to develop a strategic framework for the domestication and the genetic improvement of French algal aquaculture through an integrated approach combining stakeholder mapping, assessment of genetic management practices and knowledge transfer. The main objective is to co-design, for each species, a genetic roadmap for domestication and genetic improvement to strengthen the resilience of production facilities and culture collections, improve production quality and enhance adaptation to future challenges. In parallel, technical workshops and training sessions will be organized for professionals. Public outreach activities, including conferences, trade fairs and social media communication, will also be organised to improve societal awareness and perception of domestication and genetic improvement approaches in algaculture.

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Action plan

The first phase consists of an exhaustive inventory of algal production facilities and culture collections operating in France.

Two working groups “macroalgae” and “microalgae, including spirulina” were created to gather together scientific, technical and industry stakeholders. These working groups will provide shared and comprehensive summaries of current knowledge on genetic improvement for the most important species, and for specific issues related to individual species. In parallel, a mapping of the stakeholders, and associated value chains will be conducted, to identify opportunities and weaknesses of these emerging new sectors, to design scenarios, and to qualify potential environmental, societal and socio-economic risks for the future roadmap of these value chains.

Expertise in reproductive biology and genetics in algae and quantitative genetics in animals will be combined with companies’ know-how to design selective breeding programs according to species.

Based on those findings, each working group will produce an operational roadmap identifying technological bottlenecks, research and innovation priorities, and the most appropriate genetic improvement strategies according to the biological, economic, organizational and regulatory characteristics of each sector.

Expected outcomes

By the end of the project, TEHASIS will deliver a roadmap to initiate domestication and genetic improvement within French algal production facilities and culture collections while increasing awareness among professionals of the benefits of genetic management and selective breeding. By promoting the adoption of genetic tools and concepts, the project aims to make these approaches more accessible, thereby enhancing the competitiveness, innovation capacity and resilience of the sectors, including social acceptance.

TEHASIS also seeks to strengthen collaboration among algal farmers, scientists and technical stakeholders by establishing a national network dedicated to algaculture genetics and promoting knowledge exchanges and innovations.

In the longer term, the project will support the establishment of sustainable private and public technical and scientific support. The knowledge generated, operational roadmaps and collaborative network developed through TEHASIS will help identify sector-specific research priorities and stimulate future applied research and innovation projects. Beyond its national scope, TEHASIS may serve as a model for the development of coordinated breeding strategies across Europe, fostering collaboration between research institutions, industry stakeholders and policymakers to strengthen the sustainability, competitiveness and resilience of the European algae sector.

Acknowledgment

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ASSESSING COASTAL SUSTAINABILITY THROUGH MACROALGAL COMMUNITIES ON MOROCCO'S CENTRAL ATLANTIC COAST

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Introduction:

Marine macroalgae are major primary producers in shallow coastal ecosystems and are widely recognized as sensitive indicators of environmental quality, ecological status, and habitat change. Because macroalgal assemblages respond rapidly to variations in nutrient availability, hydrodynamics, light, substrate stability, and pollution, they are increasingly used in coastal monitoring frameworks to assess ecosystem condition and sustainability (Harley et al., 2012; Mancuso et al., 2021). Seaweeds are not only important for the environment, but they also have increasing socio-economic value. They help support fisheries, contribute to the blue bioeconomy, and are used in the food and industries. They also play an important role in supporting the livelihoods of coastal communities. For this reason, conserving and monitoring seaweeds is important for both biodiversity management and sustainable development. (FAO, 2021; UNCTAD, 2024; Valero et al., 2017). On the central Atlantic coast of Morocco, these issues are especially important because coastal productivity is strongly shaped by upwelling dynamics, hydrodynamic forcing, and local geomorphology (Belmajdoub et al., 2023; Makaoui, et al., 2023). Recent work on the Moroccan Atlantic margin shows that upwelling intensity varies markedly in space and time and plays a central role in nutrient enrichment, phytoplankton productivity, and the distribution of marine organisms. In the Agadir–Cap Ghir sector, coastal circulation is further influenced by semidiurnal tides and dominant westerly to northwesterly winds, creating a physically dynamic environment where benthic communities can provide valuable ecological signals.

On the central Atlantic coast of Morocco, coastal productivity is strongly influenced by upwelling dynamics, hydrodynamic forcing, and local geomorphology. Studies conducted along the Moroccan Atlantic coast have shown that upwelling intensity varies markedly across space and time and plays a central role in nutrient enrichment, phytoplankton productivity, and the distribution of marine organisms (Belmajdoub et al., 2023; Makaoui, et al., 2023). In the Cap Ghir area, coastal circulation is further influenced by semidiurnal tides and dominant westerly to northwesterly winds, creating a physically dynamic environment in which benthic communities can provide valuable ecological signals (Rhomad et al., 2022). Although ecological importance of this coastline, the knowledge of Atlantic Moroccan macroalgae is still limited at the local level. Recent studies from El Jadida and the southern Atlantic coast have shown that Moroccan shores contain a high diversity of macroalgae (Benhniya et al., 2024; Boutafda et al., 2025). However, the presence and distribution of these species are still not well documented in several areas. This lack of information is particularly important for Cap Ghir, where intense hydrodynamics movement and permanent upwelling create favorable conditions for the development of diverse macroalgal communities, but where local baseline data are still scarce. The study aims to assess macroalgal biodiversity, evaluate their role as indicators of coastal ecosystem health and sustainability on the central Atlantic coast of Morocco.

Material and methods

Study area

The study focused on the intertidal zone of the Cap Ghir site, located on the central Atlantic coast of Morocco.

Sampling and Identification

Macroalgae samples were collected across different seasons. Species were identified based on standard morphological and anatomical criteria using existing taxonomic keys for the region.

Data Analysis

The community structure was analyzed by calculating:

The Qualitative Dominance Index (DQi), which assesses the relative species richness of the major taxonomic groups.

The Rhodophyceae/Phaeophyceae (R/P) ratio, used to infer the thermal affinity of the flora according to Feldmann (1937).

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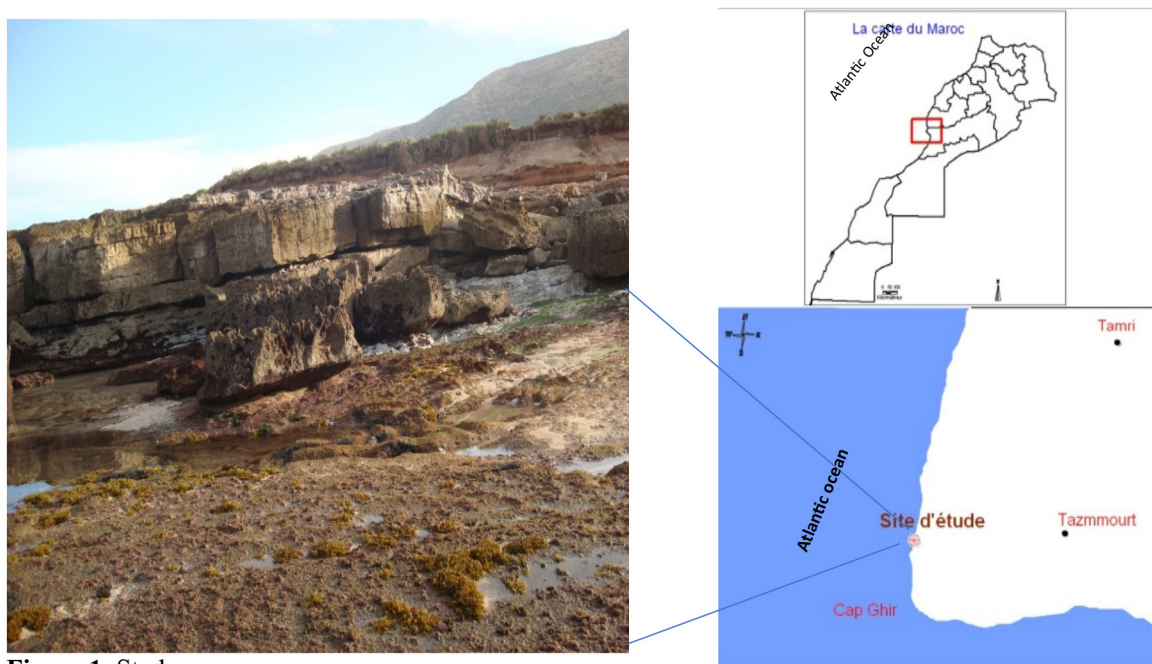


Figure 1: Study area

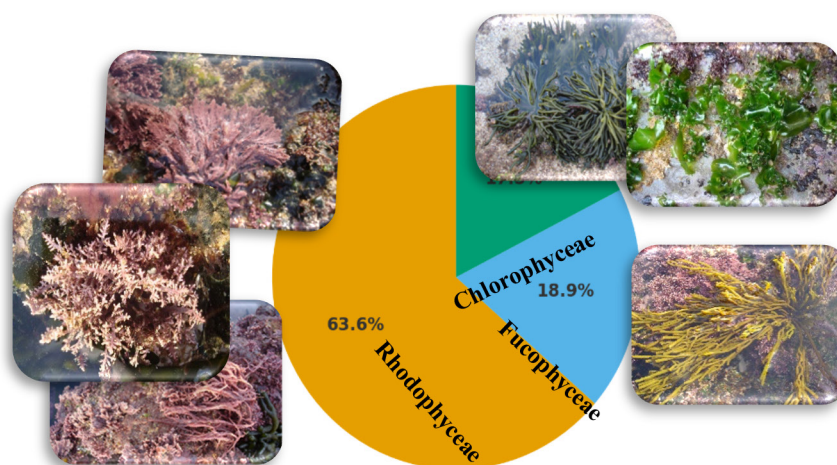


Figure 2: Qualitative dominance of different systematic groups

Results & Discussion

The floristic inventory of the Cap Ghir region identified 76 species, distributed into three classes:

Qualitative Dominance: The floristic composition shows a strong dominance of red algae (63.6 %), confirming that Cap Ghir hosts a highly diverse and stable macroalgal community. This dominance is often linked to stable and favorable local environmental conditions (Hassoun et al., 2018).

Thermal Affinity (R/P Ratio): The calculated R/P ratio is an indicator of the flora's thermal affinity (Boudouresque & Verlaque, 2013).

$$R/P = 48 \text{ (Rhodophyceae)} \div 15 \text{ (Phaeophyceae)} = 3.2$$

A value of $R/P = 3.2$ indicates a flora of warm-temperate affinity (Feldmann, 1937). This finding is consistent with the known hydrodynamics and seasonal variability of the central Moroccan Atlantic coast (Bessa et al., 2021). Overall, this study provides a crucial baseline for future environmental monitoring.

(Continued on next page)

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EPIGENETIC DIFFERENCES BETWEEN LACUSTRINE AND RIVERINE BROWN TROUT FINGERLINGS REVEALED BY NANOPORE SEQUENCING

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Introduction

Brown trout (*Salmo trutta*) exhibit striking life-history polymorphism with lacustrine (lake-dwelling) forms typically growing faster and reaching larger sizes, while maturing later, when compared to riverine residents. Neutral genetic differences between these ecotypes are minor, suggesting that epigenetic regulation could underpin their divergent phenotypes. DNA methylation mediates phenotypic plasticity and may transmit parental environmental information to offspring. Epigenome-wide studies in salmonids show that maternal life history has a stronger influence on offspring methylation patterns than early-life temperature, with differentially methylated regions (DMRs) enriched for genes involved in metabolism, neurobiology and digestion [1]. Genome scans of migratory versus resident brown trout highlight outlier loci near genes such as FAM70A, GRID1, CDH20 and EZH2 [2], while rainbow-trout smolts show DMRs in genes controlling neuronal differentiation (NEUROD4, GRM1), muscle development (MYOM3) and immune function (RSAD2) [3]. These findings provide a framework for investigating epigenetic differences between lacustrine and riverine fingerlings.

Materials and methods

Ripe brown trout were collected from Lake Bled and the Sava Bohinjka River during the 2018 spawning season, and their progeny reared at same conditions, forming novel broodstock of both ecotypes. In spawning season 2024, broodstock were striped and milked, producing F2 lacustrine and riverine progeny. At six months of common garden conditions, five fingerlings from each ecotype were sampled. High-molecular-weight DNA was extracted from brain and liver tissues using a modified CTAB protocol and sequenced on MinION Mk1c devices (R10.4 flow cells). Base-calling and methylation detection used Guppy and Megalodon. Methylation frequencies were mapped to the *S. trutta* genome, and DMRs between ecotypes were identified with MEDIPS and methylartist. Genes overlapping significant DMRs were annotated and analysed for functional enrichment.

Expected outcomes

Because lacustrine trout grow faster, we anticipate DMRs in promoters of metabolic and muscle-development genes, leading to enhanced energy assimilation and growth. Differences may also occur in neurodevelopmental and immune-related genes, reflecting adaptations for adfluvial movement and stress responses. Candidate targets include FAM70A, GRID1, CDH20, EZH2, NEUROD4, GRM1, PCDHGC5, CLDN10, MYOM3 and RSAD2 [2][3]. These epigenetic signatures will be validated through targeted resequencing and gene expression analyses in subsequent work.

Discussion

This study will be among the first to apply long-read nanopore sequencing to examine genome-wide methylation differences in brown trout. Comparing lacustrine and riverine fingerlings raised in a common garden will help disentangle inherited epigenetic marks from environmental effects. Identifying DMRs in growth- and migration-related genes may explain the fast growth of lacustrine forms and aid development of markers for selective breeding for producers to select or induce beneficial epigenetic states. Understanding epigenetic contributions to life-history variation also informs conservation by highlighting the importance of maintaining epigenetic diversity.

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COMPARATIVE STRATEGIES FOR YEAR-ROUND PRODUCTION OF STRIPED BASS (*Morone saxatilis*)

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Introduction and approach

Striped bass (*Morone saxatilis*) is a high-value finfish species with expanding potential in North American aquaculture, including pond-based and recirculating aquaculture systems. However, year-round production remains constrained by a narrow spring spawning window, seasonal availability of fry and fingerlings, and temperature-dependent growth. This synthesis compares two complementary strategies for stabilizing supply: staggered broodstock conditioning for out-of-season spawning and cold banking with feed restriction for temporary storage of fingerlings. The comparison evaluates biological control points, infrastructure needs, operational complexity, economic implications, animal welfare considerations, and suitability for hatchery, grow-out, and vertically integrated production systems. It also frames ongoing techno-economic assessment as a necessary next step for comparing these approaches across production scales and infrastructure pathways.

Results and implications

Staggered broodstock conditioning. Broodstock manipulation acts at the reproductive control point by using photoperiod, temperature, and, when needed, hormonal induction to phase-shift maturation and spawning. Reproductive timing is governed by the brain–pituitary–gonad axis, and by exposing multiple broodstock cohorts to phase-shifted photothermal regimes, hatcheries can induce spawning outside the natural spring window to provide predictable egg and larval supply (Andersen et al., 2021). However, this approach requires controlled indoor facilities, skilled technical labor, and substantial energy inputs for temperature regulation. It also carries risks of variable gamete quality, spawning failure, and additional juvenile-rearing challenges when larvae are produced outside the season when earthen pond nursery systems are biologically feasible.

Cold banking and feed restriction. Fingerling cold banking acts later in the production cycle by reducing temperature and ration to slow metabolism and preserve juveniles for delayed stocking. For striped bass, a practical cold-banking range is approximately 10–15°C, with careful rewarming and refeeding to support compensatory growth—a well-described response mediated by growth hormone and insulin-like growth factor signaling (Borski et al., 1998; Picha et al., 2006; Turano et al., 2007). Sorting fingerlings into groups undergoing one to four weeks of feed restriction can extend availability by several weeks. This strategy is less capital-intensive and more accessible to grow-out operations, but extended or poorly managed storage may compromise growth recovery, immune function, welfare, and overall production efficiency.

Integration and economic assessment. The two strategies should be viewed as complementary rather than mutually exclusive. Hatcheries can use staggered spawning to expand the availability window for larvae and fingerlings, while producers can selectively cold bank cohorts for several weeks to align stocking with tank availability, seasonal conditions, and market timing. A hybrid approach offers a resilient framework for improving domestic striped bass supply chains. A critical gap identified in this review is the absence of detailed economic analyses comparing these strategies across production scales and system types. Ongoing techno-economic modeling work (Brawley, in preparation) is directly addressing this need, estimating capital and operating costs, energy sensitivity, scale effects, and market-aligned stocking scenarios so that biological feasibility can be translated into practical decision tools for hatcheries, growers, and vertically integrated operations.

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THE ROLE OF MARITIME SPATIAL PLANNING IN SUPPORTING EGD-DRIVEN AQUACULTURE DEVELOPMENT IN THE MEDITERRANEAN

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The European Green Deal (EGD) establishes a cross-sectoral agenda directly impacting on the marine environment, with a momentum which has been recently reinforced by the Ocean Pact (European Commission, 2025). Maritime Spatial Planning Directive (MSPD, 2014/89/EC) can directly contribute to the achievement of EGD sectoral goals, and methodologies assessing the relevance of this contribution are currently of interest (Bocci et al., 2026).

The present work explores the relationship between MSPD implementation and aquaculture spatial planning, through Allocated Zones for Aquaculture - AZA - definition and management (Macias et al., 2019). The work was carried out within the project MEDIGREEN “Mediterranean approach towards a maritime European Green Deal in MSP” funded by EMFAF (EMFAF-2023-PIA-MSP). We investigated the role of MSP in supporting sustainable aquaculture development by performing two parallel analyses:

1. collecting information from aquaculture experts and management bodies on the current stage of development of AZA and MSP planning in the Mediterranean, on the level of integration between AZA sectorial plans and MSP plans, and on the availability/interoperability of support tools used in aquaculture planning. Prioritized areas and areas with high potential for aquaculture currently identified in the different countries were also mapped;
2. performing a desk-based analysis on three aspects which are influencing aquaculture spatial planning and management: i) multi-level governance alignment; ii) interaction of aquaculture with other maritime uses and with marine conservation; iii) environmental interactions and climate resilience of aquaculture zones. This second step was performed by reviewing aquaculture strategic documents at the national level, technical documents from European Commission and General Fisheries Commission for the Mediterranean, and by collecting examples from existing AZA experiences.

Results of the two analyses are discussed, in order to define more clearly the role of MSP in contributing to the EGD objective of sustainable sea-food production; the discussion benefits from the feedback received within two recent meetings of the Community of Practice on Maritime Spatial Planning in the Mediterranean (MED-MSP COP), in which the preliminary results of the work were presented.

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ADVANCED OXIDATION PROCESSES FOR REMOVAL OF OFF-FLAVOUR COMPOUNDS IN RECIRCULATING AQUACULTURE SYSTEMS (RAS)

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Recirculating aquaculture systems (RAS) often experience accumulation of off-flavour compounds, notably geosmin and 2-methylisoborneol (MIB). The complexity and variability of both the RAS system and its water matrix, including organic matter and salinity, challenge the effective design and application of treatment technologies for off-flavour control.

This research demonstrates advanced oxidation processes (AOPs) for the removal of geosmin and MIB in RAS. A laboratory-scale comparative study was conducted using water from a commercial salmon RAS growout system to assess the degradation of geosmin and MIB using UV/H₂O₂, O₃/H₂O₂, vacuum-UV, and ozone pre-treatment followed by vacuum UV. The laboratory results were used to derive degradation kinetics and compare treatment performance under realistic RAS matrix conditions. Selected AOP configurations were subsequently tested at pilot scale to verify the laboratory findings.

A mechanistic mass-balance model was developed to describe geosmin and MIB dynamics in RAS growout and purge tanks and to enable application across different system designs. The model represents compound production, distribution between fish and water, and hydraulic exchange, and incorporates water-treatment from the laboratory- and pilot-scale experiments.

The model allows sizing and operational assessment of AOP water treatment to reduce geosmin and MIB in water and fish. Depending on the case, treatment can be applied in growout to prevent offflavour and avoid depuration, increasing capacity and avoiding weight loss. Alternatively, treatment during depuration can reduce purge time and thereby minimize weight loss.

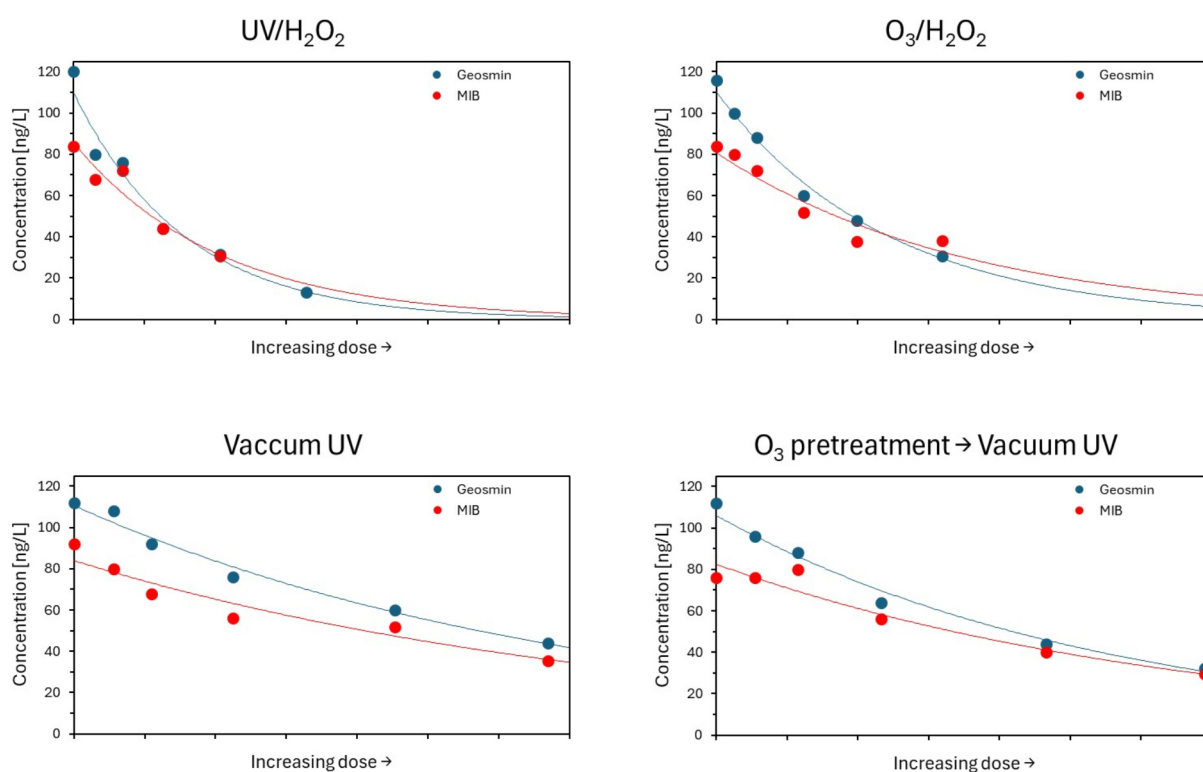


Figure 1 Comparative test of four advanced oxidation process (AOP) for degradation of geosmin and 2-methylisoborneol (MIB) in commercial salmon RAS water: UV/H₂O₂, O₃/H₂O₂, vacuum-UV (VUV), and ozone pre-treatment followed by vacuum-UV (VUV). Water concentrations are shown as a function of increasing dose.

SEASONAL VARIATION IN FATTY ACID COMPOSITION OF EUROPEAN SEA BASS (*Dicentrarchus labrax* L.) FILLETS AND FEED

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Introduction

Dietary lipids provide energy and essential nutrients, including essential fatty acids. Long-chain polyunsaturated fatty acids (LC-PUFA), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), support fish growth, development and regulation of inflammatory processes. Marine fish have a limited capacity to synthesize LC-PUFA from alpha-linolenic acid (ALA) and linoleic acid (LA); consequently, tissue fatty acid composition is strongly influenced by dietary supply, in addition to species, age and rearing conditions (Carr et al., 2023). Seasonal changes in feed raw-material availability may alter dietary fatty acid profiles and potentially affect fillet composition. This study compared total fat and selected fatty acid indicators in feed and European sea bass (*Dicentrarchus labrax*) fillets collected during winter and summer 2025.

Materials and methods

European sea bass (400-600 g) were sampled from commercial cages during the first (Q1, winter) and third (Q3, summer) quarters of 2025. Fish were fed at approximately 80% of apparent satiation with diets containing 43% crude protein and 16-20% crude fat. Fillet samples were prepared as 12 pooled samples, each comprising five fish, and fatty acid composition was determined. Twelve feed samples were collected in each quarter. Fatty acid composition was expressed as a percentage of total fatty acids (%TFA).

Two-way analysis of variance (ANOVA) was performed using quarter (Q1 and Q3) and sample type (feed and fillet) as factors.

Results

Total fat was lower in fillets than in feed in both Q1 (9.12 vs 16.92 g/100 g sample) and Q3 (10.20 vs 18.28 g /100 g sample). In Q3, ALA was lower in fillets than in feed (4.06 vs 6.96 %TFA), whereas the Q1 values were 4.13 and 5.57 %TFA, respectively. In Q1, EPA was lower in fillets than in feed (3.35 vs 4.14 %TFA); Q3 values were 3.30 and 3.71 %TFA. The EPA/DHA ratio was also lower in Q1 fillets than in Q1 feed (0.56 vs 0.70), while Q3 values were 0.55 and 0.65. The n-3/n-6 ratio was numerically lowest in Q1 fillets (0.71), compared with 0.91 in Q1 feed, 0.78 in Q3 fillets and 0.83 in Q3 feed. No significant differences in LA or DHA proportions between fillets and feed were detected in either season. Within fillets, total fat and the evaluated fatty acid indicators remained stable between Q1 and Q3.

Discussion

The seasonal stability of fillet fat content and the evaluated essential fatty acid profile suggest that the feeding strategy maintained a consistent nutritional composition of European sea bass fillets across winter and summer. The lower ALA proportion in Q3 fillets relative to feed may reflect increased metabolic utilization during the warmer period. Lower EPA and EPA/DHA values in Q1 fillets relative to feed, together with the comparatively low n-3/n-6 ratio in winter fillets, may indicate greater utilization or lower retention of n-3 fatty acids during colder conditions. These findings suggest that increasing the winter dietary supply of n-3 fatty acids, particularly EPA, could be considered. The feed fatty acid profile remained comparatively stable between sampling periods, indicating that seasonal variation in raw materials did not substantially affect the evaluated feed composition.

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FIRST EVALUATION OF *Pogonias courbina* REARED IN BIOFLOC ENRICHED WITH *Nannochloropsis oculata*: EFFECTS ON GROWTH PERFORMANCE, HEMATOLOGICAL PARAMETERS, AND IMMUNE RESPONSES

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Introduction

Brazilian marine fish farming still has low production, despite the high market value of this sector. In this context, native species with aquaculture potential, such as *Pogonias courbina*, require further investigation. Biofloc technology (BFT) has become established as a more sustainable production system capable of contributing to water quality maintenance and providing microbial biomass as a supplementary nutrient source. Supplementation of BFT with microalgae, such as *Nannochloropsis oculata*, may enhance its potential nutritional and immunological benefits for cultured organisms. Therefore, this study evaluated, for the first time, the effects of biofloc technology, with and without *N. oculata* supplementation, on the growth performance, hematological parameters, and immune responses of juvenile *P. courbina*, using a clear-water system as the control.

Materials and Methods

The experiment was conducted over 42 days at the Federal University of Santa Catarina, using a completely randomized design with four treatments: clear water in continuous flow (CW), bioflocs (BFT), BFT supplemented with 5 mg L⁻¹ of *N. oculata* (BFT+5), and BFT supplemented with 10 mg L⁻¹ of *N. oculata* (BFT+10). Each treatment was evaluated in quadruplicate in 400 L tanks containing seawater at 33 ppt, and tanks assigned to the biofloc treatments were inoculated with mature biofloc at 20% of the total water volume. Twenty juveniles were stocked per tank and fed a commercial diet containing 40% crude protein and 7% ether extract three times daily (08:00, 12:00, and 16:00 h) to apparent satiation. The microalgae, obtained from a live culture, were added twice weekly. Water quality was monitored throughout the experiment; sucrose was added when ammonia levels exceeded 1 mg L⁻¹, and solids were removed by settling when total suspended solids reached 400 mg L⁻¹. Water temperature was maintained between 27 and 28 °C, and dissolved oxygen remained above 5 mg L⁻¹. At the end of the experiment, *Vibrio alginolyticus* was used to assess plasma antimicrobial and agglutination activities through twofold serial dilution assays.

Results and discussion

Juvenile *P. courbina* adapted well to the BFT conditions, with survival rates approaching 100% across all treatments. Fish reared in the CW system exhibited significantly higher final body weight and weight gain than those reared in the biofloc treatments ($p < 0.05$), while BFT+5 showed intermediate responses for some production parameters. No significant differences were detected among treatments for the hematological or immunological parameters evaluated, although the BFT+10 treatment showed a tendency toward lower erythrocyte and leukocyte counts.

Similar findings were reported by Silva et al. (2023), who observed increased final biomass in *Oreochromis niloticus* when the biofloc system was supplemented with 5 mg L⁻¹ of *Scenedesmus obliquus*. The authors likewise found no overall differences in hematological parameters. However, unlike the findings of the present study, microalgal supplementation increased monocyte counts and antimicrobial titers. Although *N. oculata* contains bioactive compounds with potential antimicrobial and immunostimulatory properties (Zahran et al. 2023), the contrasting responses may be related to interspecific differences in biofloc utilization, since *O. niloticus* is recognized as an active consumer of particles in the system, whereas this ability is not yet well established for *P. courbina*. Furthermore, the lower values observed for some hematological parameters in the BFT+10 treatment suggest that increasing the microalgal concentration may not result in a dose-dependent immunostimulatory response.

(Continued on next page)

Table 1. Growth performance and nutritive efficiency indices of *P. courbina* juveniles reared for 42 days.

Parameter	CW	BFT	BFT+5 mg L ⁻¹	BFT+10 mg L ⁻¹	p-value
Initial weight (g)	23.0 ± 0.51	22.5 ± 0.39	22.9 ± 0.77	23.3 ± 0.49	0.336
Final weight (g)	51.71 ± 2.04 a	44.71 ± 2.12 b	45.98 ± 1.51 b	43.50 ± 2.76 b	0.001
Final biomass (g)	1021.3 ± 48.75 a	894.2 ± 42.30 b	919.6 ± 30.26 ab	860.0 ± 72.72 b	0.006
Weight gain (g)	28.7 ± 1.77 a	22.2 ± 2.41 b	23.1 ± 1.64 b	20.2 ± 3.01 b	0.002
SGR (% day ⁻¹)	1.9 ± 0.07 a	1.6 ± 0.15 b	1.7 ± 0.11 ab	1.5 ± 0.18 b	0.006
FCR	1.3 ± 0.03	1.5 ± 0.10	1.4 ± 0.06	1.6 ± 0.17	0.054
PER	1.9 ± 0.04 a	1.7 ± 0.11 ab	1.8 ± 0.07 ab	1.6 ± 0.17 b	0.046
Survival (%)	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	98.8 ± 2.50	0.473

Values are presented as mean ± standard deviation (SD).

SGR = specific growth rate; FCR = feed conversion ratio; PER = protein efficiency ratio.

Table 2. Hematological and immunological parameters of *Pogonias courbina* juveniles reared for 42 days.

Parameter	CW	BFT	BFT+5 mg L ⁻¹	BFT+10 mg L ⁻¹	p-value
Erythrocytes (×10 ⁶ μL ⁻¹)	3.05 ± 0.25	3.02 ± 0.25	3.21 ± 0.14	2.66 ± 0.29	0.065
Thrombocytes (×10 ⁴ μL ⁻¹)	1.80 ± 1.06	2.11 ± 0.70	1.37 ± 0.68	1.47 ± 0.50	0.574
Leukocytes (×10 ⁵ μL ⁻¹)	1.69 ± 0.34	1.60 ± 0.38	1.42 ± 0.13	1.05 ± 0.25	0.052
Total immunoglobulin (mg L ⁻¹)	34.9 ± 5.6	34.4 ± 5.8	32.5 ± 8.8	37.2 ± 6.5	0.808
Total protein (mg L ⁻¹)	44.1 ± 5.7	42.9 ± 6.9	42.9 ± 7.1	46.8 ± 6.1	0.815
Antimicrobial plasma titer (Log2)	3.0 ± 0.0	3.5 ± 0.6	3.0 ± 0.0	3.0 ± 0.0	0.498
Agglutination titer (Log2)	2.5 ± 1.0	3.0 ± 1.4	2.7 ± 1.2	1.8 ± 1.0	0.098

Values are presented as mean ± standard deviation (SD).

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PRODUCTION OF MUGILID (*Mugil cephalus*) USING BIOFLOC TECHNOLOGY (BFT): NUTRITIONAL AND ENVIRONMENTAL STRATEGIES

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Introduction

Biofloc Technology (BFT) is an alternative to conventional aquaculture systems that converts nitrogenous waste into microbial biomass, thereby improving nutrient recycling within the culture environment. The flathead grey mullet, *Mugil cephalus*, is an important member of the family Mugilidae and is widely distributed in saline and euryhaline waters, including the Mediterranean region. Its omnivorous and detritivorous feeding behavior allows the direct utilization of biota present in the culture environment, making this species a suitable candidate for rearing under BFT conditions. Given the rich microbial community characteristic of biofloc systems, different nutritional and environmental strategies may be applied to further optimize fish performance and physiological responses. Based on this premise, three experiments were conducted to investigate different nutritional approaches and management in *M. cephalus* reared under BFT. Specifically, the objectives were to: (1) evaluate the early intestinal physiological response to dietary fishmeal replacement; (2) assess the effects of continuous darkness, probiotic supplementation, and their interaction on zootechnical performance; and (3) evaluate the effects of microalgae and probiotic supplementation on stress responses under oxygen and density challenges.

Materials and Methods

Three independent experiments were conducted with juvenile *Mugil cephalus* reared under BFT conditions. In Experiment 1, three diets containing 15%, 5%, or 0% fishmeal (**FM15**, **FM5**, and **FM0**, respectively) were tested in triplicate for 27 days, followed by evaluation of growth performance, body composition, intestinal enzyme activity, gut microbiota, and transcriptomic responses. In Experiment 2, fish were exposed for 72 days to a 2 × 2 factorial combination of photoperiod (12 h light:12 h dark vs. continuous darkness) and probiotic supplementation, resulting in four treatments: **Light**, **Dark**, **Light+Prob**, and **Dark+Prob**. Zootechnical performance was evaluated, together with changes in the bacterial community of the biofloc between the beginning and end of the trial. In Experiment 3, four treatments were tested in triplicate for six weeks: **Dark**, **Dark+Prob**, **Microalgae**, and **Micro+Prob**. The Dark groups were maintained under continuous darkness, whereas the Microalgae groups received *Chlorella vulgaris* under a 12 h light:12 h dark photoperiod. Probiotic supplementation was applied to the Prob treatments, and stress responses were subsequently assessed under oxygen and density challenges. The microalgae, obtained from a live culture, were added once a week, at 18×10^6 cells ml⁻¹. In experiments 2 and 3, 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, and the probiotic (Sanolife PRO-W *Bacillus subtilis* and *B. licheniformis*) was added once a week, at 1g m⁻³. Water quality was monitored throughout the three experiments.

Results and discussion

Across all three experiments, survival was close to 100% in all treatments, and fish maintained satisfactory growth, supporting the suitability of biofloc technology for rearing juvenile *M. cephalus*. In experiment 1, complete fishmeal replacement maintained growth and feed efficiency but was associated with reduced intestinal enzyme activity, lower lipid reserves, and transcriptomic changes related to lipid metabolism. Regarding the intestinal microbiota, beta diversity showed significant differences between the initial and final samples (FM15: $p = 0.009$; FM0: $p = 0.007$), while no differences were detected between the dietary treatments ($p = 0.798$), suggesting a temporal shift potentially influenced by the biofloc microbial community. Biofloc nutritional composition was not significantly affected by the different diets. In Experiment 2, continuous darkness and probiotic supplementation significantly improved SGR and relative weight gain, while probiotic supplementation also increased protein efficiency ratio, and no significant interaction between the two factors was detected. Biofloc maintained under continuous darkness, and therefore under more heterotrophic conditions, showed a significantly higher crude protein content than biofloc exposed to light. The microbial community in the water was mainly influenced by time. Beta-diversity analysis revealed a clear temporal shift in community composition ($p = 0.001$), whereas

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photoperiod, probiotic supplementation, and their interaction had no significant effect. In the third trial, weekly microalgae supplementation improved zootechnical performance under light conditions compared with Experiment 2, resulting in values comparable to those observed in the Dark and Dark+Prob treatments. Microalgae supplementation was associated with a more favorable stress response, as CAT activity was maintained during the challenge, GPx activity returned to basal levels after 24 h of recovery, and the cortisol increase was lower than in the Dark group. Compared with the previous experiment, crude protein content increased in the Microalgae treatment to values comparable to those observed under the Dark condition, whereas the Micro+Prob treatment remained lower than the Dark. Overall, Experiments 1 and 2 confirmed that the microbial community changed over time as the biofloc system matured, consistent with previous reports by Kim et al. (2022). The higher biofloc protein content observed under continuous darkness is consistent with Martinez-Porchas et al. (2020), who reported markedly higher protein levels under heterotrophic than photoautotrophic conditions. The inclusion of microalgae in the biofloc system in Experiment 3 did not improve growth performance, however, its potential functional role was evident, which is consistent with Jung et al. (2017) in tilapia. These results suggest that even under BFT conditions, *M. cephalus* showed limited tolerance to complete fishmeal replacement, whereas it adapted well to different environmental and management strategies within BFT without compromising productive performance.

Acknowledgment

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NEW NORDIC “AOSA”: LAND-BASED AQUACULTURE OF DANISH *Ulva*

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Seaweed cultivation can serve as an innovative solution to recycle nutrients in effluent from land-based aquaculture systems, and here, the green algae genus *Ulva* is a promising candidate due to its high phenotypic plasticity, high growth rates, and biochemical composition. The qualities of *Ulva* have been described worldwide; however little research and development has been conducted in the Nordic countries. This study presents an extensive screening and characterisation of Danish *Ulva* strains and their performance in a range of temperatures, nutrient regimes and salinities. Ten Danish/German *Ulva* strains were documented to grow 6-22% day⁻¹ in free floating cultures, of these, several strains of *U. compressa*, showcased the most stable growth performances (11-32% day⁻¹). Additionally, the optimal temperature for nitrogen uptake in *U. compressa* was repeatably identified to be around 14-16 °C (30-40 mg L⁻¹ week⁻¹) while tissue concentrations of high value compounds such as ulvan were higher at colder temperatures (150-180 mg g⁻¹). The salinity tolerance of *Ulva* strongly depended on the nutrient availability, and the growth rate at low salinities decreased 10-fold for *U. fenestrata*, while salinity (5-25 ppt) did not affect growth of neither *U. fenestrata* nor *U. compressa* when nutrient concentrations were high. These results are currently being applied at a land-based salmon production site where the growth of *U. compressa* is grown in effluent water. Hence, this extensive research and development approach will showcase the potential to integrate *Ulva* cultivation in a range of aquaculture facilities and hereby diversify the product portfolio, while increasing both profit of the production.

MARINE MULTI-USE – COPRODUCTION OF RENEWABLE ENERGY AND REGENERATIVE BLUE FOOD IN SCANDINAVIAS LARGEST OFFSHORE WINDFARM

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Sharing the space at sea

Pressure on the coastal zone and offshore waters in Europe is increasing, in particular as a consequence of the expansion of offshore wind farms (OWF) [1]. At the same time we look to the sea for future food production to reduce pressure on land. With the recent UN Biodiversity Beyond National Jurisdiction Treaty and the EU Ocean Pact, the international focus on protection and restoration of the sea calls for responsible governance and management structures. Marine Multi-Use (MU), where space, infrastructure and services are shared between several actors [2] is suggested for 1) Optimising use of ocean space, 2) Boosting efficiency, reducing footprints and increasing safety of operations, and 3) Enabling the circular blue economy with the potential to increase global low trophic aquaculture (LTA) production by 132% using 10% of existing and planned OWF area for production of seaweed and mussels [3]. Realising this potential, however, requires adaptive governance frameworks that recognise both engineered ecosystem services and disservices across ecosystems, marine activities and value chains [4].

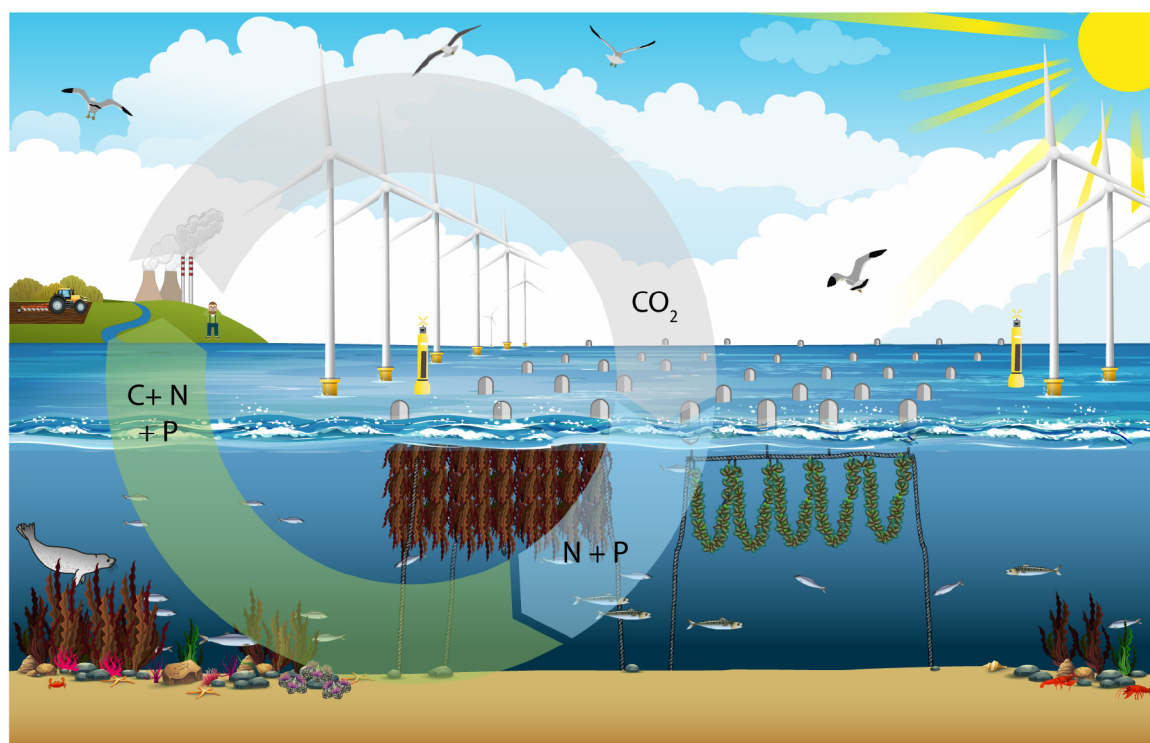


Figure 1. Concept figure of the Multi-Use prototype installed and run over 3 years in the Danish Krieger Flak Windfarm [3]. In the windfarm, production of mussels and seaweed take up nutrients and carbon, and the blue biomass is used for food, feed or biomaterials. The removal of nutrients mitigates eutrophication.

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Outcomes of the WIN@sea project

Since 2023, a pilot scale lowtrophic aquaculture (LTA) site in the OWF at Danish Kriegers Flak (DKF) in the Baltic Sea has been established, operated and decommissioned as part of the the WIN@sea and OLAMUR projects. Here, joint MU procedures between OWF operator and LTA producer was demonstrated: site selection, risk assessments, shared wind and wave forecasts, joint maintenance operations and Crew Transfer Vessel (CTV) undertaking regular monitoring of site status, environmental parameters and biodiversity. Low salinity limited the seaweed productivity at DKF, but mussel settling and growth showed potential for production of marine protein. Dynamic 3D ecological models were used for predicting yields and ecosystem services and further taken into a site selection tool for identifying optimal sites for LTA within OWF, considering also local carrying capacity. Based on project data, LifeCycle Analysis is in progress for documenting the environmental and economic viability of the MU concept in the Danish Baltic and North Sea basins. The concept and dilemmas of sharing the space at sea has been developed into a board game for communicating Marine MU and ocean literacy in the Danish education system.

Acknowledgment

The project WIN@sea constitutes one of three case studies in the EU Mission Ocean Lighthouse project OLAMUR. The project is supported by the VELUX FOUNDATION, Aage V. Jensen NATURFOND and the European Union, grant no. 101094065. WIN@sea and OLAMUR embrace the EU Mission Ocean Charter.

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POSTPRANDIAL DYNAMICS OF FREE ARGININE IN THE DORSAL MUSCLE OF *Oncorhynchus mykiss* STRAINS

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Introduction

Arginine is a functional amino acid involved in protein synthesis, nitrogen metabolism and growth regulation in fish (Li et al., 2021). Different color phenotypes of rainbow trout (*Oncorhynchus mykiss*) may be associated with distinct metabolic responses due to their genetic background (Hattori et al., 2020). Therefore, this study evaluated postprandial changes in free arginine concentration in the dorsal muscle of four rainbow trout strains subjected to fasting and refeeding.

Materials and Methods

Four rainbow trout strains (wild-type, cobalt blue, yellow-albino and white-albino) were reared under identical conditions and fed a commercial diet containing 45% crude protein. After 48 h of fasting, fish were sampled at 0, 15, 180 and 360 min after refeeding. Free arginine concentrations in dorsal muscle were determined by HPLC-UV, reverse-phase column at 35°C, using 10 mM sodium acetate buffer and acetonitrile in gradient mode, with a flow rate of 1 mL min⁻¹, according to Badawy and Morgan (2010) and Matheus et al. (2017). Data were analyzed using a generalized linear mixed model with Gamma distribution and log link function, considering the interaction between time and strain.

Results and Discussion

Under fasting conditions, the cobalt blue strain showed the highest arginine concentration (Figure). After feeding, arginine levels changed differently among strains, with the white-albino strain presenting the highest concentration at 15 min postprandially. However, this strain showed the most pronounced decline over time and the lowest concentration at 360 min. In contrast, wild-type and cobalt blue trout maintained higher arginine concentrations throughout the experimental period ($p < 0.05$). These results indicate that genetic background influences arginine metabolism in rainbow trout. Previous studies have shown that pigmentation traits are associated with differences in stress responsiveness, endocrine regulation and growth performance (Khan et al., 2016; Rodríguez et al., 2019). The higher arginine availability observed in the more pigmented strains may reflect differences in amino acid utilization, whereas the marked reduction observed in the white-albino strain suggests a greater allocation of arginine to physiological maintenance processes, potentially reducing its availability for muscle growth.

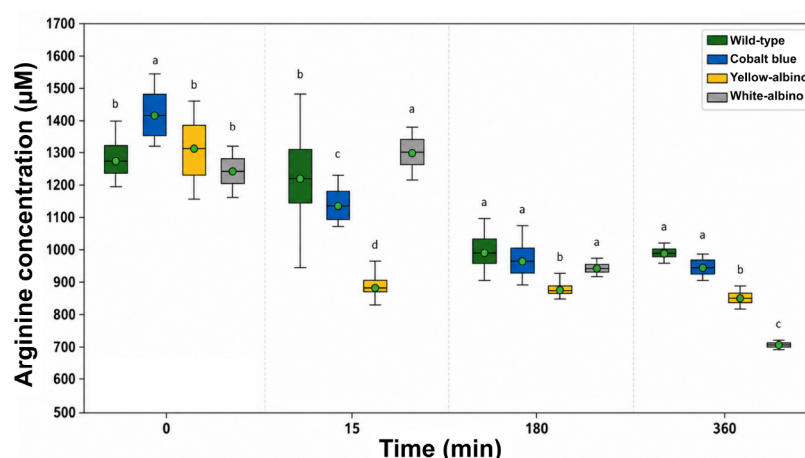


Figure. Free arginine concentration (µM) in the dorsal muscle of rainbow trout strains after refeeding. Values represent means $\pm$ SD. Different lowercase letters indicate significant differences among strains within each sampling time ($p < 0.05$).

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INTEGRATED MULTI-TROPHIC AQUACULTURE FOR NUTRIENT-NEUTRAL SYSTEMS: LIFE CYCLE ASSESSMENT AND NUTRIENT MASS-BALANCE EVIDENCE

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Introduction

Aquaculture expansion is increasingly required to meet rising demand for aquatic foods, but continued growth depends on reducing environmental pressures, particularly nutrient emissions in sensitive coastal systems (Crona et al., 2023). Integrated multi-trophic aquaculture (IMTA) has been proposed as a strategy to mitigate aquaculture-derived nitrogen (N) and phosphorus (P) emissions by co-cultivating extractive species such as mussels and seaweed alongside fed finfish (Chopin et al., 2012; Buck et al., 2018). However, the biomass scale required to achieve nutrient-balanced production remains poorly quantified, particularly under environmentally constrained Baltic Sea conditions (Sickander and Filgueira, 2022). This study evaluates the environmental feasibility of nutrient-neutral offshore aquaculture using a rainbow trout (*Oncorhynchus mykiss*) system integrated with blue mussels (*Mytilus edulis*) and seaweed (*Ulva* spp.), combining attributional life cycle assessment (LCA) with farm-gate nutrient mass-balance modelling.

Materials and Methods

Primary foreground data were collected from a multi-use offshore aquaculture site in Tagalaht Bay, Estonia, hosting adjacent trout, blue mussel, and seaweed cultivation systems. The assessment followed ISO 14040/14044 and applied a cradle-to-farm-gate system boundary. Nutrient emissions from trout farming were quantified using a farm-gate mass-balance approach based on feed inputs, biomass retention, and residual emissions, consistent with established aquaculture nutrient budgeting methods (Cho and Bureau, 1997). Environmental impacts were assessed in SimaPro using the ReCiPe 2016 Midpoint (H) method. Scenario analysis was used to estimate the mussel and seaweed biomass required to offset trout-derived N and P emissions under different IMTA configurations, and coefficients were further scaled to European aquaculture production levels.

Results

Per ton of harvested trout, offshore production emitted 63.7 kg N and 6.9 kg P to the marine environment, while feed production dominated climate change impacts, contributing approximately 2,880 kg CO₂-eq t⁻¹ trout. Scenario analysis indicated that achieving full nutrient neutrality would require 10.37 t of extractive biomass per ton of trout, comprising 6.76 t of mussels and 3.61 t of seaweed. In contrast, the current pilot IMTA configuration offset only about 0.42 % of N and 0.45 % of P emissions. Scaling these requirements to current European trout production suggests that nutrient-neutral production would require extractive biomass volumes far beyond present seaweed production capacity.

Discussion

The results show that IMTA can reduce eutrophication impacts by converting nutrient losses into harvestable biomass, but nutrient-neutral aquaculture is strongly constrained by the scale at which extractive species must be deployed. Under Baltic Sea conditions, current pilot-scale IMTA systems fall far short of delivering ecologically meaningful nutrient compensation (Buck et al., 2018; Sickander and Filgueira, 2022). At the same time, the analysis reveals a structural divergence between nutrient and climate mitigation pathways: while IMTA can reduce farm-level eutrophication, feed production remains the dominant driver of climate and resource-related impacts, consistent with previous aquaculture LCA studies (Henriksson et al., 2012). Overall, the study provides system-level evidence that nutrient-neutral aquaculture will likely require a dual strategy combining ecologically significant extractive biomass deployment with upstream feed optimization.

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MONOCULAR ZERO-SHOT ANOMALY DETECTION FOR EARLY DISEASE WARNING IN EUROPEAN SEABASS

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Introduction

Aquaculture disease surveillance requires continuous and non-invasive tools capable of detecting changes in fish welfare before mortality becomes evident. European seabass (*Dicentrarchus labrax*) is a high-value species in Mediterranean aquaculture, and bacterial outbreaks such as vibriosis can cause both behavioural deterioration and visible body changes. In this study, it is investigated whether monocular underwater video provides early disease-related evidence without requiring fish handling, invasive sampling, or a disease-specific visual model.

A zero-shot approach is adopted, in which potential disease-related anomalies are identified by comparison with the behavioural and visual patterns observed in the remaining healthy fish population. It is hypothesised that disease pressure alters two complementary signal families: how fish move and use the available space, and how individual fish appear in segmented image crops. By combining these complementary signals, this study demonstrates that disease-related changes are detected up to two days before mortality becomes evident.

Materials and Methods

The experimental setup consisted of 154 healthy European seabass monitored in a controlled tank measuring $1.33 \times 1.03 \times 0.58$ m. Two underwater cameras with a resolution of 1280×864 pixels and a frame rate of 30 fps were used. The experimental protocol combined progressive thermal stress, in which the water temperature was increased from 23°C to 30°C, over eight weeks and rapidly returned to 23 °C during week nine. The pathogen was exposed at this final week to induce vibriosis-related stress.

Videos were recorded 24h/day and subsequently trimmed at specific times, including four feeding periods of 8 minutes each, and 1 minute videos in intervals of one hour between feedings. The most relevant footage for this study was the period immediately after feed delivery (at 07:30, 12:00, 15:00, and 16:45).

Fish instances were isolated from the tank background, and masks, bounding boxes, and cropped images were obtained on a frame-by-frame basis using SAM 3 video segmentation (Carion et al., 2025). Two separate methods were then applied, and their outputs combined to generate the final warning signal.

For Method A, instance masks were converted into trajectories by extracting mask centroids and linking detections across consecutive frames. A Hungarian assignment step based on centroid distance and mask overlap was applied (Kuhn, 1955; Bewley et al., 2016). Short, low-confidence, or physically implausible tracklets were discarded before the remaining trajectories were aggregated into descriptors of spatial occupancy, residence time, transition frequency, movement persistence, and swimming activity (Anas et al., 2020).

For Method B, segmented crops were used to compare two alternative physical-evidence representations. The first representation consisted of DINOv3 embeddings (Siméoni et al., 2025) classified using a gradient-boosting model (Friedman, 2001). The second consisted of handcrafted descriptors based on colour, texture, morphology, deformation, and posture-related cues.

Finally, the outputs of both Method A and B were combined to identify relevant indicators, reduce potential false positives, and increase confidence in the final warning signal.

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Results

Method A reveals disease-related changes in how fish move through the tank, including alterations in spatial use, movement persistence, residence patterns, and overall swimming activity, especially evident during feeding periods. Healthy fish display vigorous swimming and rapidly approach the surface with fast and explosive movements in search of food. In contrast, diseased fish tend to remain near the bottom, separated from the rest of the shoal, and exhibit slow and subdued swimming.

Method B shows strong internal discrimination between healthy and vibriosis-labelled fish crops. The first approach combines frozen DINOv3 embeddings with a gradient-boosting classifier, achieves an ROC AUC of 0.9454 for crops extracted from videos in which the anomaly is known to be present. As a second approach, an interpretable model based on handcrafted colour and texture features achieves a comparable ROC AUC of 0.9447 under the same internal test conditions. The selected alternative for Method B is the DINOv3 representation, while the handcrafted features were used as an interpretable baseline (Ahmed et al., 2022). The most relevant physical indicators include reduced chromatic variation, altered texture, and deviation from the compact appearance manifold associated with healthy fish.

The aggregation of crop-level predictions at the video level makes the physical signal easier to interpret. For each video, the proportion of fish crops classified as suspicious is calculated for each video. This proportion is consistently lower for the healthy videos. The difference in proportions observed reaches 0.660, indicating a clear separation between the two groups in the current test set. In pre-mortality videos, abnormal visual patterns are detected up to two days before mortality. Overall, the trajectory-based and crop-based methods provide complementary evidence: the former captures changes in fish behaviour and spatial use, while the latter detects changes in physical appearance from the same monocular video recordings.

Discussion

The main contribution of this study is a dual-evidence, zero-shot early-warning framework for detecting disease-related signals in aquaculture. The proposed system combines behavioural anomalies derived from fish trajectories with physical anomalies extracted from segmented fish images.

Trajectory analysis (Method A) captures changes in movement, activity, and use of space (Arechavala López et al., 2025, Yang et al., 2021). This source of evidence is particularly valuable because behavioural deterioration may emerge before severe external signs or mortality become apparent. Crop-level analysis (Method B) complements this information by identifying visible changes in the bodies of individual fish, including alterations in colour, texture, morphology, and posture.

The combination of Method A and Method B increases confidence that an alert reflects genuine biological deterioration. Agreement between the two methods is particularly important because individual image features may be affected by factors such as temperature, camera angle, tank appearance, or illumination, while trajectory estimates may be influenced by occlusions, missed detections, or non-fish artefacts.

In all videos in which an anomaly is indicated by Method A, typically one or two days before mortality, the same anomaly is also successfully detected by Method B, and vice versa. A high level of consistency is therefore observed across all tested videos containing diseased fish. More than two days before mortality, abnormalities may be detected independently by either method, depending on the position and visibility of the diseased fish.

The current performance metrics should be interpreted as preliminary internal evidence. Further validation should include balanced temperature conditions, open-sea cages, variable turbidity and illumination, greater levels of occlusion, and larger fish populations. Future work should also investigate the advantages of using multiple cameras. A multi-camera configuration may improve detection performance and reduce false alarms caused by tracking errors, occlusions, or non-fish artefacts.

Acknowledgment

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TRANSPORT STRESS IN FARMED FISH: SKIN MUCUS AND TISSUE MULTIOMICS IN ATLANTIC SALMON, EUROPEAN SEABASS, AND RAINBOW TROUT

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Introduction

Fish transport is an essential yet stressful practice in aquaculture. Acute transport-induced stress can compromise both specific and non-specific immunity, disrupt osmoregulation, and increase disease susceptibility, potentially leading to delayed mortality. Monitoring fish welfare physiologically remains challenging, as current methods rely on invasive procedures such as blood sampling, tissue collection, or surgical implantation of biosensors. Moreover, the established indicators are often detected too late for timely intervention. The skin and mucus of teleost fish are central to innate and adaptive immunity and homeostasis. Given the feasibility of minimally invasive or non-invasive sampling, they represent promising matrices for biomarker discovery enabling earlier and less intrusive health monitoring in farmed species.

Materials and methods

To investigate the molecular signatures following acute stress, three key European aquaculture species: Atlantic salmon (*Salmo salar*), European seabass (*Dicentrarchus labrax*), and rainbow trout (*Oncorhynchus mykiss*) were subjected to a transport challenge. The transportation trials were designed to simulate current transport conditions specific to each species. Skin tissue and mucus samples were collected at three time points: before transport, immediately after stress exposure, and following a 24-hour recovery period. Bulk polyA RNA sequencing was conducted on skin samples, while small RNA sequencing and proteomics were performed on skin mucus. Sequencing data were processed using nf-core pipelines for RNA-seq (nf-core/rnaseq) and small RNA-seq (nf-core/smrnaseq), while proteomic data were analysed using the FragPipe pipeline. Differential expression analysis (DEA) was performed using edgeR (transcriptomic) and FragPipeAnalystR (proteomic) R packages. Additionally, unsupervised machine learning based on Multi-Omics Factor Analysis (MOFA+) was performed. The results of DEA and MOFA were compared and similarities highlighted.

Results and Discussion

Differential expression analysis, complemented by MOFA multiomics integration, revealed species-specific stress responses differing in both timing and magnitude. Enrichment analyses and shared element comparisons further highlighted the negative physiological impact of transportation stress across species. The acute transcriptomic response, based on the number of differentially expressed genes immediately following stress, was markedly stronger in Atlantic salmon (3,134 DEGs) and rainbow trout (1,241 DEGs) compared to European seabass (309 DEGs). Conversely, European seabass exhibited a stronger and more rapid skin mucus proteomic response, being the only species with differentially abundant proteins detected at the post-stress timepoint. Cross-species comparison identified a conserved set of 42 genes and 3 miRNAs while no protein was found to be commonly differentially abundant across all three species. To filter out candidate biomarkers, elements consistently identified across at least four subgroups defined by species, pairwise comparisons, and omic layers, were highlighted. The 17 highlighted biomarker candidates are primarily associated with coagulation, extracellular matrix remodelling, wound healing, and immune response which was also reflected in the observed commonly activated pathways, while pathways associated with biosynthesis and metabolism were inhibited. Among the candidates, arginase (ARG2), ferroxidase (F5), and papilin a (PAPLNA) were the most consistently represented across subgroups. Independently, MOFA integration identified factors that separated samples by condition in all three species. While several candidate biomarkers were recovered across species and omic layers, none were consistently top-weighted across all three species in the factors relevant to stress, emphasising the complexity of the stress response and the need for multiple complementary discovery approaches. Future analyses incorporating supervised machine learning and expanded datasets will further refine biomarker prioritisation and improve fish welfare assessment.

Acknowledgment

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WHAT DRIVES THE ADAPTATIVE SUCCESS OF THE FISH PATHOGEN *Edwardsiella piscicida*?

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Introduction

Edwardsiella piscicida is a major bacterial pathogen affecting aquaculture worldwide. Although comparative genomics has revealed substantial diversity within the species, the functional significance of lineage-specific accessory genes remains largely unexplored. As the accessory genome represents a major source of genomic diversity, its characterization may provide valuable insights into the evolutionary processes underlying bacterial adaptation. Here, we investigated the functional repertoires encoded by lineage-specific accessory genes across *E. piscicida* lineages.

Material and Methods

Ten *E. piscicida* isolates representing different geographical origins and fish hosts were subjected to whole-genome sequencing using Illumina technology by EgoGenomics. Raw sequencing reads were quality assessed before de novo genome assembly. The resulting assemblies showed genome completeness ranging from 91% to 97%. Genomes were structurally and functionally annotated prior to comparative pangenome analysis. A core-genome phylogeny was reconstructed from the core gene alignment to define the major evolutionary lineages. Lineage-specific hypothetical proteins identified within the accessory genome were functionally characterized using eggNOG-mapper to infer their putative biological roles.

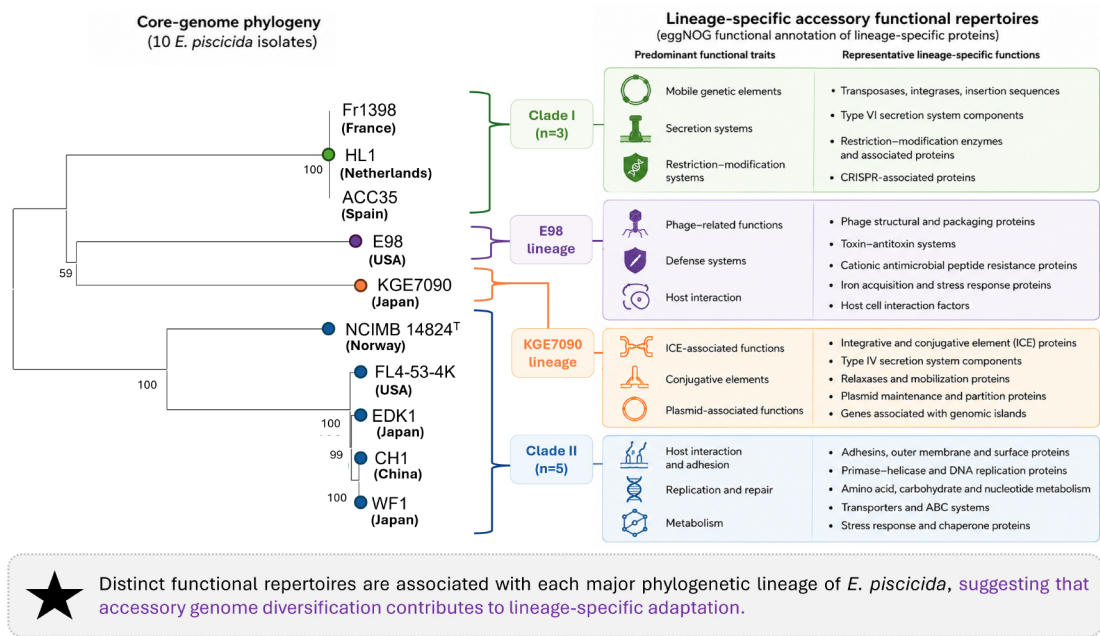


Figure 1. Core-genome phylogeny of the ten *E. piscicida* isolates included in this study and summary of the predominant lineage-specific accessory functional repertoires inferred from eggNOG annotation. Comparative analysis identified distinct functional repertoires associated with each major phylogenetic lineage, suggesting that diversification of the accessory genome may contribute to adaptive success of *E. piscicida*.

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Results and Discussion

The pangenome comprised 4,372 gene clusters, including 2,946 core genes and 1,426 accessory genes. Whereas the core genome was highly conserved, the accessory genome contained numerous lineage-specific proteins, many of which lacked previous functional characterization. The annotation revealed that the major phylogenetic lineages differ in the composition of their accessory genomes with biological processes including horizontal gene transfer, host interaction, defense mechanisms and regulatory functions. Clade I was characterized by proteins associated with mobile genetic elements, Type VI secretion systems and restriction-modification systems, whereas the E98 lineage, KGE7090 lineage and Clade II displayed representative functions related to bacteriophages and defense systems, conjugative elements, and adhesion, replication and metabolism, respectively (Figure 1). These findings suggest that the evolutionary diversification of *E. piscicida* is accompanied by functional specialization of its accessory genome, consistent with the hypothesis that lineage-specific accessory functional repertoires may contribute to the adaptive success of this pathogen in aquaculture environments.

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AI-DRIVEN DIGITAL TOOLS SUPPORTING THE ALGAE AQUACULTURE VALUE CHAIN UNDER GLOBAL CHANGE: The AlgaeProBANOS Digital Ecosystem

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Introduction

Aquaculture is expected to play an important role in addressing global challenges such as climate change, limited resources, and the growing demand for sustainable food and bio-based products. In this context, algae cultivation is becoming increasingly relevant, as it can provide biomass for food, feed, pharmaceuticals, cosmetics, and other applications, while also contributing to circular bioeconomy models.

At the same time, working with algae is not straightforward. Information about species, cultivation conditions, processing technologies, and market applications is scattered across many different sources, including scientific papers, databases, and industry reports. This makes it difficult for stakeholders to get a clear overview and to make informed decisions, for example when selecting suitable species, identifying cultivation locations, or evaluating production strategies.

In the [AlgaeProBANOS](#) project, we address this problem by developing a set of digital tools that bring these different types of information together and support more data-driven decision-making across the algae value chain.

A Digital Ecosystem for the Algae Value Chain

The tools developed in Work Package 4 are designed to work together as a small ecosystem, following a simple pipeline from data collection to analysis and decision support.

At the base, we developed automated data collection and integration tools that gather information from different sources, including open-access scientific publications, Wikipedia, biodiversity databases, marine observation systems, and project-specific datasets. The goal here is not just to collect data, but to clean, structure, and connect it in a way that makes it usable across the other tools.

On top of this data layer, we built the [Algae Knowledge Base](#), which allows users to explore algae-related knowledge through a simple question–answer interface. The system uses a retrieval-based approach, meaning that it looks up relevant scientific sources before generating an answer. This makes the output more reliable and traceable compared to general AI tools, and better suited for domain-specific questions such as cultivation conditions or application areas.

The interactive [Algae Farming and Products dashboard](#) provides a more visual way of exploring the data. Users can browse species, look at their geographic distribution, identify producers, and explore possible product applications. In practice, this makes it much faster to answer questions that would otherwise require searching across multiple databases and sources.

Finally, the [Algae Economist](#) focuses on the economic side. It allows users to define simple production scenarios and get an estimate of costs and key indicators such as LCOP, ROI, and IRR. This helps in getting a first idea of whether a certain production pathway is economically interesting or not.

All tools described here are publicly available and can be accessed online at <https://algaeprobanos.eu/digital-tools/>.

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Results and Impact

When used together, these tools support a simple but practical workflow. Users can start by exploring species and regions in the dashboard, then use the Knowledge Base to better understand the biological and technical aspects, and finally use the Economist to check whether a given scenario could make economic sense. This allows stakeholders to:

- identify promising species and locations
- access relevant knowledge more quickly
- connect biological data with industrial applications
- and perform basic economic assessments

Based on initial use within the project, the main benefit is a clear reduction in the time needed to gather and connect information, together with better transparency in how decisions are made.

Conclusions

The tools developed in AlgaeProBANOS show how digitalisation and AI can support more informed and structured decision-making in algae aquaculture. Instead of relying only on fragmented data and experience, stakeholders can use integrated tools that combine data collection, knowledge access, visual exploration, and economic analysis.

This becomes particularly important in the context of global change, where faster and more reliable decisions are needed to develop sustainable and scalable aquaculture solutions.

Acknowledgment

This work was conducted within the framework of the **AlgaeProBANOS project** – “*Accelerating algae product development in the Baltic and North Sea*”, which has received funding from the European Union’s Horizon Europe research and innovation programme under grant agreement No. 101112943.

EFFECTS OF DIETARY CHLORELLA VULGARIS ON FEED EFFICIENCY AND FLESH PROXIMATE COMPOSITION IN JUVENILE STERLET (*Acipenser ruthenus*)

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Chlorella vulgaris is a protein-dense green microalga with functional properties relevant to aquaculture nutrition. This study evaluated the influence of graded dietary inclusion of *C. vulgaris* dried powder on growth performance, feed efficiency, and flesh proximate composition of juvenile sterlet (*Acipenser ruthenus*) under recirculating aquaculture system (RAS) conditions. A 36-day feeding trial was conducted with four dietary groups: Control (standard commercial diet) and three *C. vulgaris* inclusion levels — V1 (1.5%), V2 (3.0%), and V3 (4.5%) — with 100 fish per tank (initial mean weight 1.59 ± 0.52 g). Specific growth rate (SGR) ranged from 7.39 to 7.58%/day in supplemented groups versus 6.71%/day in Control, and feed conversion ratio (FCR) improved with inclusion, with V2 achieving the best value (0.382 vs. 0.483 in Control). Differences in final body weight and length were not statistically significant ($p > 0.05$). Flesh composition differed significantly among groups for all measured parameters: crude protein increased with inclusion level, fat content peaked in V2, and ash content increased progressively with *C. vulgaris* dose. *C. vulgaris* powder at 1.5–4.5% inclusion significantly modified sterlet flesh proximate composition and numerically improved growth performance and feed efficiency, with the 3% inclusion level identified as the most feed-efficient under the conditions of this study.

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MYCOBIOME AND BACTERIAL MICROBIOTA OF MEAGRE (*Argyrosomus regius*) FED POULTRY BY-PRODUCT MEAL-BASED DIETS

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Introduction

Processed animal proteins, such as poultry by-product meal (PBM), combine high nutritional quality with a low environmental footprint, aligning with circular bioeconomy principles (Galkanda-Arachchige et al., 2020). Meagre (*Argyrosomus regius*), a high-value whitefish species native to the Adriatic Sea, has gained renewed interest in Mediterranean aquaculture due to its quality, rapid growth, and adaptability to farming conditions (Kružić et al., 2016). Beyond growth and economic performance, evaluating how alternative protein sources shape the gut microbial ecosystem is essential, since intestinal bacteria and fungi play key roles in digestion, nutrient absorption, and immune homeostasis, and can serve as sensitive early indicators of dietary impact on fish health. This study presents the first combined assessment of the bacterial (16S rRNA) and fungal (ITS2) intestinal communities of meagre fed increasing dietary inclusion levels of PBM as a fishmeal substitute.

Material and methods

Three experimental diets incorporating PBM as a partial fishmeal replacement at two inclusion levels (T1, T2), plus a fishmeal-based control diet (T3/CF), were formulated to meet the nutritional requirements of meagre. 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) were acclimated for four weeks on a commercial diet before being randomly distributed into six concrete tanks (600 L; 18 fish per tank, two replicate tanks per treatment). At the conclusion of the trial after one year, digesta content was sampled from the proximal (PI) and distal (DI) intestine of fish from each dietary group for gut microbial community characterization (12 replicates per treatment for each intestinal segment). Fungal (ITS2) and bacterial (16S rRNA V3-V4 region) community composition were characterized in parallel by amplicon sequencing on the Illumina platform. Sequences were denoised into amplicon sequence variants (ASVs) using DADA2 and taxonomically classified against the SILVA 138.2 (16S) and UNITE (ITS2) reference databases. Alpha and beta diversity were calculated for both datasets, and group differences were tested using ANOSIM and PERMANOVA (Bray-Curtis).

Results and discussion

The fungal (ITS2) community was diverse and highly individually variable, dominated by Malasseziomycetes, Dothideomycetes, Sordariomycetes, Eurotiomycetes and Saccharomycetes. Alpha diversity was consistently higher in the proximal than distal intestine, and the fishmeal control showed the lowest fungal diversity and evenness in both segments, while beta diversity and ANOSIM/PERMANOVA confirmed that dietary effects on the mycobiome were strongest distally, with T2 showing an intermediate community structure between T1 and the control.

The bacterial (16S) community was overwhelmingly dominated by Gammaproteobacteria across nearly all samples and diets, indicating a comparatively stable core bacterial assemblage relative to the fungal community. In contrast to the mycobiome, bacterial alpha diversity was highest, not lowest, in the fishmeal control, particularly in the distal intestine. Beta diversity, ANOSIM and PERMANOVA converged on a single, striking pattern: the distal intestinal bacterial community of the control group was markedly and significantly distinct from all other diet-by-segment combinations, whereas proximal communities and both PBM-fed groups (T1, T2) remained largely indistinguishable from one another regardless of gut segment.

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Taken together, both microbial kingdoms indicate that PBM inclusion reshapes the meagre intestinal microbial ecosystem primarily in the distal intestine, but with contrasting directionality: PBM diets increased fungal diversity and evenness relative to the control, while the bacterial community of PBM-fed fish appeared more homogeneous than the compositionally distinct, more diverse distal bacterial community observed under the fishmeal-based diet. These complementary bacterial and fungal signatures underscore the value of a dual-kingdom amplicon approach for capturing diet-driven shifts in fish gut microbial ecology, and support poultry by-product meal as a viable, sustainable fishmeal alternative without inducing a uniformly destabilizing effect on the meagre gut microbiota.

Acknowledgment

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FUNCTIONAL LOW-ENERGY DIET WITH INULIN IMPROVES PERFORMANCE AND PHYSIOLOGICAL ADAPTATION OF EUROPEAN SEA BASS (*Dicentrarchus labrax*) UNDER CHRONIC THERMAL STRESS

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Introduction

Marine aquaculture is increasingly challenged by rising water temperatures, particularly in the Mediterranean, where temperatures close to 30 °C may induce chronic thermal stress in European sea bass (*Dicentrarchus labrax*). Temperature-dependent growth responses in fish have been widely described (Fonds et al., 1992). In parallel, functional ingredients such as inulin may contribute to intestinal homeostasis, microbiota stability and innate immune modulation under intensive farming and heat stress conditions (Srirengaraj et al., 2023; Zhou et al., 2022). The present study evaluated the effects of three diets differing in protein and lipid levels, dietary energy content, and inulin inclusion on growth performance, immunology, histopathology, welfare, and fillet proximal composition of *D. labrax* reared under chronic thermal stress.

Materials and Methods

A 12-week nutritional trial was conducted in a recirculating aquaculture system using 450 *D. labrax* specimens with an initial body weight of 113.24 ± 15.82 g. Fish were randomly distributed into 9 tanks (50 fish tank⁻¹) in triplicate groups. Water temperature was progressively increased from 22 to 30 °C during the first week and then maintained at 30.0 ± 1.0 °C. Three diets were tested: i) diet CD, commercial diet (46.5 % protein, 20 % lipid); ii) diet HE, high-energy diet (44 % protein, 24 % lipid); and iii) diet FD, functional diet (46.5 % protein, 18 % lipid) supplemented with 0.5 % inulin. Productive performance was assessed through final body weight, specific growth rate, feed intake, feed conversion ratio and survival. Immunological analyses included reactive oxygen species in plasma, lysozyme activity in plasma and mucus, protease/anti-protease activity and total proteins in mucus, and phagocytic activity in anterior kidney. Histopathology was evaluated in liver and intestine. Welfare assessment included plasma glucose, triglycerides, cholesterol and cortisol, together with hepatic triglycerides, cholesterol, superoxide dismutase, catalase, glutathione peroxidase and malondialdehyde. Final fillet proximal composition was also determined.

Results

Initial body weight was similar among treatments. At the end of the trial, fish fed diet FD showed the highest final body weight (209.23 ± 6.23 g fish⁻¹) and specific growth rate (0.75 ± 0.04 % day⁻¹), significantly higher than fish fed diet HE (191.84 ± 1.93 g fish⁻¹; 0.64 ± 0.01 % day⁻¹; $P < 0.05$), while diet CD presented intermediate values (204.07 ± 6.46 g fish⁻¹; 0.72 ± 0.04 % day⁻¹). Feed conversion ratio did not differ among treatments (1.82–1.83), and survival was 100 % in all groups. Most immune indicators followed similar trends among diets and did not show significant differences at the final sampling, except for phagocytic activity, which was higher in fish fed diet HE. Welfare assessment revealed significantly higher plasma glucose and triglyceride levels in fish fed diet HE, whereas fish receiving diets FD and CD displayed a more favourable metabolic profile under prolonged exposure to 30 °C. Histopathological evaluation revealed normal lipid vacuolization in liver and preserved intestinal integrity, with no relevant lesions associated with any diet. Final fillet proximal composition was not significantly affected by dietary treatment.

Discussion

The results indicate that increasing dietary lipid to 24 % was not advantageous under chronic heat stress, as the high-energy diet was associated with poorer growth performance and a less favourable metabolic response. In contrast, the functional diet supplemented with inulin provided the best overall response, combining the highest growth with lower plasma glucose and triglycerides levels and maintaining histological safety and final fillet quality. The tendency towards greater antioxidant enzyme activation in fish fed diet HE also suggests higher oxidative pressure in this group. Overall, moderate lipid levels combined with functional ingredients, such as inulin, appear to improve physiological adaptation of *D. labrax* specimens to prolonged exposure to high water temperature.

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Acknowledgment

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SURFACE MICROBIOTA DYNAMICS IN A NEW RECLAIMED-WATER AQUAPONIC SYSTEM

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Introduction

Integrating aquaponics into recirculating aquaculture systems (RAS) leverages the interactions among fish, plants, and the microbiome, supporting optimized growth and a sustainable production model (Joyce et al., 2019). Within the AWARE project, the use of treated wastewater in aquaponic systems has been shown to produce safe food in accordance with European regulations (Cabo et al., 2025). Although microbial communities are recognized as key ecosystem stabilizers, their role within aquaponics systems remain insufficiently understood (Kushwaha et al., 2025). The aim of this study is to compare the temporal evolution of bacterial surface communities at three locations within RAS units operated with treated wastewater and with dechlorinated water. Through a metataxonomic approach, the study pretend to generate insights into the composition, diversity, and potential functional roles of these communities within the aquaponic system.

Methodology

Experimental system: Experiment was 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 m² of crop planting surface with capacity for 90 lettuce plants (*Lactuca sativa* Batavian Red).

Surface sampling: was conducted at the beginning of the experiment and subsequently after two months period in accordance with the procedures outlined in ISO 18593 for the assessment of both wet and dry surfaces on the vegetable growing tables (GTs), tanks (TKs) and sumps (Ss).

Microbiome characterization: bacteriome was characterised using next-generation sequencing (NGS) techniques. Following DNA extraction (PureLink™ Microbiome DNA Purification Kit, Invitrogen) and purification (OneStep™ PCR Inhibitor Removal Kit, Zymo Research), amplification and library preparation were performed using the 16S Barcoding Kit 24 V14, from Oxford Nanopore Technologies (ONT), following the standard protocol. Finally, 16S rRNA amplicon sequencing was carried out using the ONT's MinION platform, which enables real-time, long-read sequencing. The sequences generated were analyzed in the cloud served EPI2ME, from ONT, with the 16s workflow. Sequences below 800 bp and longer than 2000 bp were discarded. Finally, filtered reads were classified with minimap2 using SILVA as reference database.

Results and Discussion

The evolution of the bacterial communities in the growing tables (GTs), sump (S) and tanks (TKs) of RAS1, RAS2 and RAS3 was characterized through metataxonomic analyses. The composition of the bacterial communities was analyzed though a non-metric multidimensional scaling (NMDS) analysis based on Bray-Curtis dissimilarity distance and subsequent clusterization.

Overall, the analysis of the microbiota present on RAS surfaces clearly revealed locationdependent differences in the temporal evolution of bacterial communities throughout the experiment. At the beginning of the trial (t0), the bacterial composition detected in the GTs clustered with that found on the Ss, both characterized by a high relative abundance of *Rhizobiales Incertae Sedis*, *Rhodobacteraceae*, *Reyranellaceae*, *Hyphomicrobiaceae*, *Chitinophagaceae*, *Legionellaceae*, and *Burkholderiaceae*. Notably, members of the order *Rhizobiales* are known to participate in nutrient cycling in aquatic systems (Jia et al., 2019).

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By the end of the experiment (t2), however, the bacterial communities in the GTs were dominated by members of the family *Aeromonadaceae* across all three RAS units, together with *Enterobacteriaceae*, which reached its highest relative abundance in the GT of RAS1 (GT1). Importantly, the absence of *Aeromonas hydrophila* in the food produced within the RAS ensured product safety.

In the case of the Tks, the initial surface-associated communities formed a distinct cluster. Nevertheless, after two months (t2), the microbial communities in the RAS units operating with reclaimed water (RAS2 and RAS3) became similar to those of the Ss, showing a predominant relative abundance of *Exiguobacteriaceae*. The order *Rhizobiales* remained detectable, particularly in the Tk and S of RAS2. This pattern suggests that microbial composition at these locations is likely shaped by the extreme environmental conditions typical of the Castellana region during summer, as members of *Exiguobacterium* are known to withstand stressors such as high temperature, salinity, and heavy metal exposure.

By the end of the trial, the microbiota on Ss and Tks in systems using reclaimed water converged, being dominated by *Exiguobacteriaceae*. In contrast, the plant GTs of all three RAS units exhibited a distinct microbial trajectory, with *Aeromonas* becoming dominant (up to 91% relative abundance). This indicates that, while reclaimed water strongly influences the bacterial communities of Ss and Tks, it has a more limited impact on those of the GTs.

Conclusion: Bacteriome evolution on Ss and Tks surfaces is influenced by the use of reclaimed water, whereas its impact on GT surfaces appears to be more limited. Differences in microbial dynamics across RAS locations may arise from systemspecific factors such as water circulation configuration, as well as the influence of biofilter, plant, and fish microbiota. Finally, considering the spatial context of each surface within the RAS, it can be inferred that submerged S and Tk surfaces more closely reflect the systemwide microbiome, whereas the exposed GT surfaces likely mirror the microbiota associated with lettuce roots.

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TOWARDS A NEW GENERATION OF INDIVIDUAL BEHAVIOUR SENSORS FOR AQUACULTURE RESEARCH

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Background

Aquaculture is increasingly driven by the need to better understand fish behaviour as a fundamental feature of health, welfare, and production performance. In cultured systems, behavioural responses emerge from the dynamic interplay between physiological state, environment and social interactions, giving rise to complex collective patterns that remain poorly resolved by conventional monitoring strategies. Current approaches to behavioural monitoring in aquaculture rely largely on population-level observations or aggregate indicators such as bulk activity, spatial distribution or group movement metrics. These methods are suitable for detecting general trends, but they provide limited insight into individual variability and may fail to resolve subtle or heterogeneous behavioural responses. This limitation is particularly relevant in experimental and pilot-scale contexts, where understanding individual responses is essential for assessing the effects of genetics, nutrition, environmental conditions or management strategies. Individual-level behaviour monitoring addresses these limitations by enabling the direct acquisition of behavioural and physiological signals from single fish. By operating at the individual scale, such approaches allow variability, heterogeneity and response dynamics to be captured, rather than averaged out at the population level. This is especially relevant in aquaculture research, where experimental questions often target differential responses to controlled changes in genetics, feeds, environmental conditions or husbandry practices. The availability of miniaturised sensing technologies has facilitated the practical implementation of individual behaviour monitoring in experimental and pilot-scale aquaculture research.

Sensor-based individual behaviour monitoring

One way to address these monitoring needs at the individual level is through sensor-based approaches. FishBIT is a miniaturized individual monitoring device designed for behavioural and welfare assessment in aquaculture research. The device is externally attached to the operculum using a minimally invasive fixation system, allowing stable placement without impairing respiration, feeding, or normal swimming behaviour. Its small size (15 x 6 x 6 mm) and low weight (below 1.8 g in air) allow fish to rapidly recover normal activity, making the device suitable for behavioural studies under experimental and controlled conditions. FishBIT integrates a triaxial accelerometer that captures opercular and body movements with high temporal resolution, enabling simultaneous recording of physical activity and respiratory responses. Opercular movements provide a reliable indicator of respiratory frequency, while body-related acceleration signals reflect locomotor activity. Both signals are acquired continuously at the individual level, without reliance on visual observation. It has been successfully applied in major European aquaculture species, including gilthead sea bream, European sea bass, rainbow trout and Atlantic salmon. Previous studies have demonstrated the robustness of the device for monitoring behavioural responses to nutritional, environmental, and physiological challenges (Calduch-Giner et al., 2022). The updated version of FishBIT (Figure 1) incorporates advances in sensor design and system integration aimed at improving usability and performance.

Battery capacity has been increased (3 V, 30 mAh), enabling extended recording periods of up to 200 hours. The hardware configuration also includes additional inertial sensing components, such as a gyroscope, expanding the range of motion-related signals available at the individual level. Connectivity has been enhanced through the integration of Near Field Communication (NFC), allowing wireless device programming and data retrieval without physical connectors. In parallel, the use of a new ultra-low-power microcontroller and updates to the internal electronics architecture have increased computational capacity and data handling efficiency, supporting more flexible signal acquisition and on-board data management.

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Future Perspectives and Development

FishBIT has been established as a reliable individual monitoring tool for assessing activity, respiratory responses and behavioural changes in aquaculture, supporting applications in welfare assessment and experimental research. Building on these capabilities, the advances presented here enhance device autonomy, connectivity and computational capacity, and extend its applicability in experimental and pilot-scale aquaculture studies. The incorporation of enhanced on-board processing capacity facilitates a more effective use of the inertial sensing hardware of the device. In this context, current research is exploring the use of gyroscopic information to support the inference of individual movement trajectories. Such trajectory-based descriptors could provide additional insight into spatial behaviour and relative positioning of fish within a group, opening new possibilities for the study of collective organisation under controlled conditions. In parallel, ongoing developments in data analysis software aim to facilitate the interpretation of complex behavioural datasets. Machine-learning-based approaches are being investigated as tools to assist in pattern recognition, data integration and decision support, facilitating the scalable use of individual behaviour monitoring in aquaculture research and precision management scenarios.

Acknowledgment

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RESULTS FROM A PILOT STUDY ON OFFSHORE MUSSEL FARMING IN THE NETHERLANDS: TECHNICAL AND ECOLOGICAL FEASIBILITY UNDER DYNAMIC CONDITIONS

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In the Netherlands, mussel farming has traditionally been concentrated in sheltered waters such as bottom plots in the Wadden Sea and the Eastern Scheldt. However, these areas are facing spatial pressures that limit further expansion. As part of the Dutch Mussel Covenant, efforts are initiated to explore the potential for alternative farming locations outside these conventional areas, including coastal areas such as the Voordelta and offshore wind farms.

These new locations offer promising opportunities to reduce pressure on the space and to enhance mussel production. On the other hand, they also present significant challenges related to environmental dynamics, technical feasibility, regulatory complexity, and financial viability. This pilot study aimed to explore the feasibility of offshore mussel farming under highly dynamic conditions.

A pilot study was conducted from 2022 to 2024, approximately 2 km offshore in the North Sea. see Figure 1. This pilot study aimed to evaluate the technical and ecological feasibility of offshore mussel farming using two different types of suspended mussel rope systems: 1) a floating double longline and 2) a submerged longline. Both systems were equipped with various rope types and sock materials to test grow-out success and mussel seed collecting during reproduction season. During the pilot phase, the system was stocked with different sizes and offspring mussels.

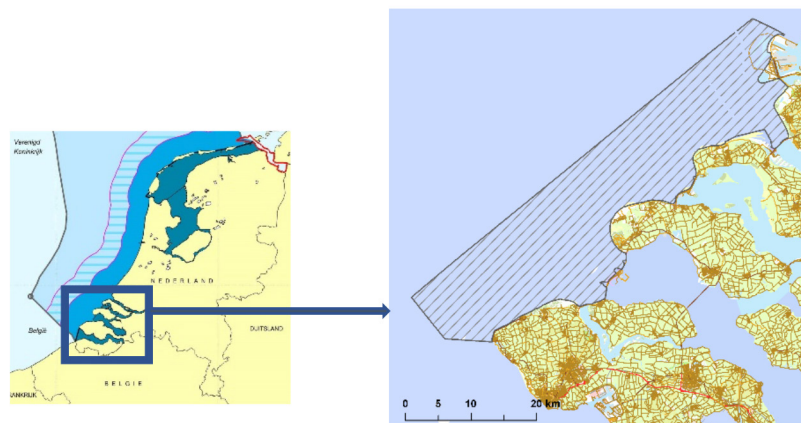


Figure 1 Pilot location in the Voordelta, the Netherlands

Given the highly dynamic water conditions at the pilot site, a broad range of parameters were monitored throughout the pilot period.

Both systems were equipped with accelerometers and load cells to track the movement of the ropes and the entire system. At the site, flow velocity profiles and wave height were measured at intervals.

Chlorophyll-a concentrations were continuously measured using sensors, both near the pilot location and at a reference site approximately 2 km away.

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Mussel growth was also monitored using a standardized method for tracking growth over time.

During the pilot period, we encountered several challenges and gained valuable insights:

Securing necessary permits was a major bottleneck.

Robust technical performance of the system, although over-dimensioned relative to actual forces.

Local seed outperformed translocated seed; growth and survival depended on depth and exposure to movement.

Operational constraints at sea limited timely management, which adversely influenced the results.

This presentation will highlight the “lessons learned” and implications for mussel farming under high dynamic conditions.

IS CO-FARMING OF AQUATIC SPECIES MORE SUSTAINABLE THAN MONOCULTURE?

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Introduction

Integrated Multitrophic Aquaculture (IMTA) has been assumed to be more sustainable than monoculture of aquatic species, but wider adoption by the industry has not yet taken off. The BLUEBOOST project investigates the extent to which adding species to monoculture systems is technically feasible, scalable and economically and environmentally more sustainable in six case studies. Here we present preliminary results of three of these case studies. The first case (Poland) is a freshwater IMTA system combining commercial carp pond farming (*Cyprinus carpio*) with crayfish (*Pontastacus leptodactylus*) and watercress (*Nasturtium officinale*) cultivation. The second case (Italy) is commercial offshore mariculture (*Sparus aurata*, *Dicentrarchus labrax*, and *Argyrosomus regius*) with the integration of flat oyster (*Ostrea edulis*) lantern farming. The third case (Brazil) is a RAS-IMTA system integrating biofloc shrimp monoculture (*Penaeus vannamei*) with oysters (*Crassostrea gazar*), sea cucumbers (*Holothuria grisea*), mullets (*Mugil liza*), and *Salicornia nei*.

Methods

Data on production and use of energy and materials, generation of waste and emissions was collected from the three case studies through site visits, and through online meetings and communication before and after the visits. Life Cycle Assessment methodology was used following a pre-defined scheme (Ziegler et al. 2024), e.g. using the functional unit 1 tonne of (mixed) biomass with an economic value and applying mass-based allocation between co-products where needed. Nutrient retention potential was assessed by quantifying the fraction of N and P retained in biomass harvested from the system relative to those released by fed species and carbon deposited and released. Basic discounted cash flow analysis was used to assess the private profitability of the proposed IMTA systems compared to the established monoculture equivalents.

Results and discussion

Important drivers of Polish carp farming were the amount of feed used per tonne of fish, which was more important than feed composition. In some years, fish were fed only grains, in others, a combination of grains and compound feed. The incremental IMTA investment is financially worthwhile due to the additional income from crayfish and watercress that can be achieved with moderate additional inputs in the form of infrastructure (crayfish cages) and labour. Still, emissions were not affected due to the small volume of additional species (a few kilos) in relation to the volume of carp harvested (25 tonnes). Also in farming of marine finfish (European seabass, gilthead seabream, and meagre) off Sardinia, environmental impacts are driven by feed use. In the IMTA setup, with the addition of European flat oyster farming, emissions remain almost unchanged, again due to the low volume of oysters farmed (96 kg liveweight oysters vs 376 tonnes of finfish). The IMTA investment is profitable, since oysters can be farmed without the use of extra feed and using relatively low-cost gear and the species added has a high economic and cultural value for which consumers may be willing to pay a premium compared to established non-native oyster species (*Magallana gigas*, Pacific cupped oyster). In the Brazilian case, emissions from shrimp monoculture were mainly driven by energy use of the recirculating system which was only moderately lowered by the addition of mullet, oysters, sea cucumbers and edible salt marsh plants (*Salicornia* sp.), again due to the low volumes of these species farmed. Although they did not require any additional feed or energy inputs, only some farming infrastructure, containers for keeping them and tubing to pump the water, the extra outputs were too small to affect the environmental assessment of the system. Whether the sales of outputs from an up-scaled IMTA operation are enough to justify the IMTA investment is still subject to inquiry.

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Conclusions

Preliminary results from the case studies confirm findings from the literature about IMTA, that the environmental benefits are small and that the main improvement potential is financial. For IMTA to deliver larger environmental benefits, the systems need to be scaled up so that volumes of co-farmed species match. As a next step of BLUEBOOST, theoretically scaled up production will be modelled, both from an environmental and economic perspective, and three more case studies will follow- in Spain, Portugal and Brazil.

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INTEGRATED ASSESSMENT OF CARBON AND NUTRIENT RETENTION: IMTA PILOT-SCALE IMPLEMENTATION WITHIN FRESHWATER AND MARINE COMMERCIAL FARMS

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Introduction

Integrated Multi-Trophic Aquaculture (IMTA) is a key strategy for enhancing resource-use efficiency and mitigating the environmental impacts of intensive monoculture by promoting nutrient (N, P, C) recirculation. The BLUEBOOST project evaluates the technical feasibility, scalability, and sustainability of integrating low-trophic species (LTS) into fed aquaculture systems across six distinct case studies. However, accurately quantifying the “retention potential” of LTS remains a methodological challenge. Existing models often rely on generalized literature-derived averages, failing to account for local and seasonal variability in farm management and species biochemical composition. This frequently leads to imprecise estimates when applied to operational, real-world systems. To address these limitations, this study presents preliminary nutrient retention results from two [BLUEBOOST project](#) case studies: a freshwater pond system (F-IMTA) in Poland (common carp, crayfish, and watercress) and a marine cage system (M-IMTA) in Italy (seabream, seabass, meagre, and flat oyster).

Methods

This study employs a stoichiometry-driven approach to quantify and retention in harvested LTS biomass (Nederlof et al., 2022), integrating laboratory diagnostics with mass-balance modeling over 7-month (F-IMTA) and 24-month (M-IMTA) cycles. and were determined via CHNS analysis, while was quantified via ICP-MS. In the freshwater IMTA daily biomass dynamics were reconstructed for crayfish and watercress. In marine oysters' daily growth was modeled via Gompertz curve fitted to weight-at-age data samplings. Fed fish species growth and metabolic outputs were simulated using TGC models and deterministic mass-balance equations (Cho & Kaushik, 1990; Wang et al., 2012). The bioremediation potential was defined as the ratio of nutrients removed by extractive components to those emitted by fed species (Checa et al., 2024).

Results

Mass balance of C, N and P values for both F- and M-IMTA inputs (feed, stocked spat/juveniles, etc.) and outputs (harvested biomasses, released emissions, etc.) were reported in table 1. Results refer to a complete production cycle: May-November 2025 for F-IMTA and May 2024-June 2026 for M-IMTA.

Discussion

Based on site-specific laboratory data across seasonal growth stages, this study provides a high-resolution, empirical snapshot of two BLUEBOOST project case studies, establishing a functional baseline for real-world IMTA bioremediation potential. Although open-system interactions, such as natural food inputs and ambient nutrient uptake, cannot be fully decoupled, these findings reflect the actual operational efficiency of the system. Ultimately, these results provide the empirical foundation required to develop commercial-scale upscaling scenarios and optimize nutrient recovery across temperate freshwater and marine multitrophic aquaculture systems.

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Table 1. Nutrient balance and bioremediation potential in F-IMTA (7 months) and M-IMTA (24 months) systems. Columns show live weights, elemental compositions (% DW), and absolute masses (kg DW) for inputs, outputs, and modelled emissions. Bioremediation potential (%) represents the ratio of nutrients retained by LTS to those released by fed species.

		Live weight	C	N	P
		kg	(%) kg d.w.	(%) kg d.w.	(%) kg d.w.
F-IMTA	Carp yield	23,269.0	(43.0) 3,456.1	(6.4) 8.6	(1.6) 94.9
	Crayfish yield	4.7	(34.3) 0.4	(6.1) 0.1	(1.2) 0.02
	Watercress yield	446.0	(75.59) 42.8	(3.7) 1.9	(0.5) 0.3
	Feed (triticale)	64,690.0	(41.9) 23,861.0	(1.6) 925.0	(0.3) 143.3
	Modelled carp emissions		25,853.0	652.2	153.3
	Bioremediation potential		(0.08)	(0.14)	(0.09)
M-IMTA	Finfish yields (seabream+seabss+meagre)	716,600.0	(43.0) 115,521.0	(8.0) 1,492.3	(2.3) 6,179.
	Oyster yield (tissue)		(38.8) 2.7	(8.3) 0.6	(0.8) 0.1
	Oyster yield (shell)	296.8	(12.3) 32.5	(0.3) 0.7	
	Formulated feed	1,844,440.0	(45.0) 810,650.0	(7.8) 131,262.0	(1.5) 24,574.0
	Modelled finfish emissions (feed loss+excretion)		696,791.7	110,079.1	18,484.2
	Shell formation CO ₂ emissions		15.4		
	Bioremediation potential		(0.003)	(0.001)	(0.0003)

Acknowledgment

This study was funded by the Sustainable Blue Economy Partnership project BLUEBOOST ID: SBEP2023-725.

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**PREDICTING THE IMPACT OF AUTOMATED FISH GROWTH MONITORING
IN SEA CAGES: ENHANCING WELFARE, ENVIRONMENTAL AND ECONOMIC
PERFORMANCE IN THE DIGITAL TRANSFORMATION OF MEDITERRANEAN
MARICULTURE**

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Introduction

Mediterranean aquaculture, composed of family-owned enterprises farming gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*), largely relies on periodic manual sampling for biomass estimation. This labor-intensive process involves crowding, anesthesia, and handling, which induces stress, scale loss, increased cortisol levels and potential impaired growth. While non-invasive AI-based machine vision is established in salmon farming, its application in the Mediterranean remains experimental and at a pilot scale. Non-invasive AI-based methods for biomass estimation are based on the use of underwater stereo-cameras that provide biomass data essential for optimizing feeding regimes, while eliminating fish handling (Anwary et al., 2023). This study models the potential environmental and economic implications of implementing high-rate image acquisition and low-error Artificial Intelligence Weight Estimation (AiWE). The system can optimize feeding protocols and reduce nutrient discharge. The goal was to investigate the extent to which AiWE reduces environmental impacts and operational costs through optimized management, while indirectly supporting animal welfare in sea-cage farming via non-intrusive monitoring and reduced acute stress.

Materials and Methods

A Life Cycle Assessment (LCA) was conducted (ISO 14040/14044) using SimaPro 10.2.0.2 and the ReCiPe 2016 Midpoint (H) and Cumulative Energy Demand v. 1.12 methods. The functional unit was 1 kg of harvested fish (seabream/seabass). Primary data (2024) were collected from a medium-scale and a small-scale Italian farm. The system boundaries (gate-to-gate) focused on activities affected by the technology shift: feed input, manual sampling days, fuel consumption, handling-related mortality, and service boat use. Three scenarios were modeled assuming feed loss reductions of 3% (S1), 5% (S2), and 10% (S3) due to improved feeding precision (i.e., reduced overfeeding), according to Chamberlain and Stucchi (2007) and Piedecausa et al. (2009). Nutrient emissions (%N, %P, and %C) were modelled accordingly. Key parameters like cage infrastructure and baseline emissions were kept constant to ensure comparability. Feed and fuel prices and labor rates were sourced from peer-reviewed literature and sectoral reports to ensure model scalability across Mediterranean aquaculture.

Table 1. LCIA baseline (per kg/fish) and scenario reductions (%). Data limited to feed and monitoring inputs; not representative of overall production impacts. BS: baseline, S1: Scenario 1, S2: Scenario 2, S3: Scenario 3. Only the impact categories of climate change (CC) and marine eutrophication (ME) are shown as an example.

	Medium-scale Farm								Small-scale Farm							
	Seabream				Seabass				Seabream				Seabass			
	BS	S1	S2	S3	BS	S1	S2	S3	BS	S1	S2	S3	BS	S1	S2	S3
CC (kg CO ₂ -Eq)	1.7	-	-	-	2.2	-	-9%	-	2.5	-	-	-	622	-	-	-
	7	4%	6%	11%	1	6%		13%	0	4%	5%	10%	7	3%	5%	10%
ME (kg N-Eq)	0.0	-	-	-	0.0	-	-	-	3.2	-	-	-		-	-	-
	4	5%	7%	13%	5	8%	10%	16%	1	3%	6%	11%	259	4%	6%	11%

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Results

Seabream production exhibited lower baseline environmental impacts than seabass across all categories (Table 1). Implementation of AiWE led to consistent impact reductions, peaking in S3 (10–15% for seabream, 10–17% for seabass). These improvements were primarily driven by reduced feed losses and avoided handling-related mortality. Under an economic perspective, AiWE would save significant labor time (27 days/year in large-scale; 15 days/year in small-scale), allowing staff to focus on higher-value tasks such as maintenance and health monitoring. At a feed price of 1.75 €/kg, AiWE scenarios yielded significant seasonal savings: for the medium-scale farm, reductions reached €241,500 (3%, for S1), €390,250 (6%, for S2), and €736,750 (11%, for S3). Small-scale savings totaled €51,647 (4%, for S1), €64,975 (5%, for S2), and €128,507 (10%, for S3).

Discussion and conclusion

This study provides the first quantitative evidence of the environmental and economic implications of implementing AI-based growth monitoring. By optimizing the Feed Conversion Ratio (FCR) through real-time weight estimation, AiWE could reduce uneaten feed waste and nutrient discharge. The transition from invasive manual sampling to non-intrusive AI monitoring enhances operational efficiency by eliminating handling-related mortality and reclaiming significant labor time. By reducing sampling labor, AiWE allows redirecting staff toward critical maintenance and inspection tasks, increasing operational efficiency. These results demonstrate that reducing physical fish handling is not only key for improving animal welfare, but also a key driver for both economic and environmental performance. AI-driven approaches should be promoted as a best practice to improve the resilience and sustainability of the Mediterranean aquaculture industry.

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CLIMATE-DRIVEN THERMAL STRESS AS AN INDICATOR OF BETANODAVIRUS OUTBREAKS IN MEDITERRANEAN GROUPERS: IMPLICATIONS FOR AQUACULTURE MONITORING AND FORECASTING

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Introduction

Ocean warming is a major driver of change in marine ecosystems, particularly in the Mediterranean Sea, where temperature increases exceed global averages. These changes are associated with more frequent marine heatwaves and can significantly alter host–pathogen interactions (Okon et al., 2024). Betanodaviruses (NNV) represent a key threat to both wild and farmed fish species. Their replication is temperature-dependent, with higher temperatures enhancing viral proliferation and disease severity. Climate change is therefore expected to increase the frequency and intensity of outbreaks mainly caused by the RGNNV genotype and the reassortant strain RGNNV/SJNNV. Recent mortality events in dusky grouper (*Epinephelus marginatus*) provide a valuable case study to investigate environmental indicators such as SST anomalies and cumulative heat stress, with implications for aquaculture monitoring and forecasting.

Materials and Methods

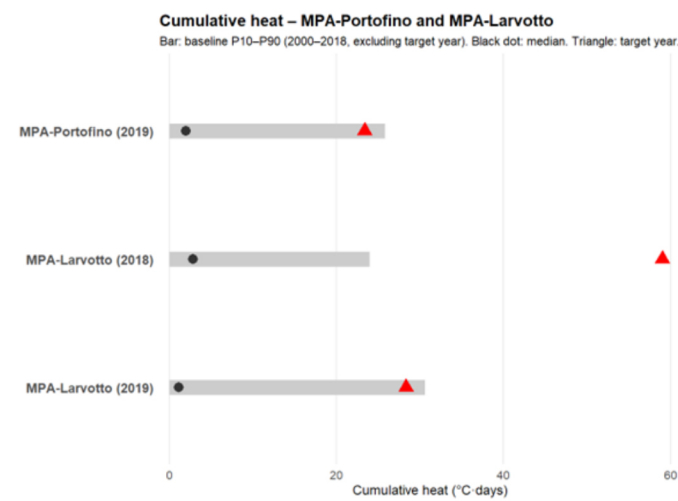
Mortality events were recorded in Portofino (Italy) and Larvotto (Monaco) Marine Protected Areas (MPAs) during 2018–2019. Virological analyses confirmed RGNNV infection. Environmental analysis was based on daily sea surface temperature (SST) data obtained from the CMEMS Mediterranean Sea Physics Reanalysis (2000–2020). SST values were spatially averaged over each MPA using all available grid cells. Two temporal windows were considered: October, corresponding to the mortality period, and the July–August–September–October (JASO) period, representing the warm season preceding outbreaks. A climatological baseline (2000–2018) was defined. For each day, climatological SST values were calculated as the mean across baseline years. Daily anomalies were obtained by subtracting climatological values from observed SST. Annual mean anomalies were calculated for both October and JASO and ranked using empirical cumulative distribution functions (ECDF) to assess their extremeness. To quantify thermal stress, a cumulative heat stress index (CHI) was calculated using the 90th percentile of climatological SST as a dynamic threshold. The index integrates both intensity and duration of threshold exceedance (°C·days), providing a robust measure of prolonged heat exposure.

Results

All analysed fish tested positive for RGNNV, with high genetic similarity among strains, suggesting regional viral connectivity. SST anomaly analysis showed heterogeneous results for October, with values close to climatological means. In contrast, the JASO period exhibited consistently elevated anomalies across sites and years. At Portofino, JASO 2019 showed an anomaly of approximately +0.9 °C (90th percentile). At Larvotto, JASO 2019 reached +1.1 °C (95th percentile), while 2018 recorded the most extreme anomaly (+1.53 °C, 100th percentile), representing the warmest season on record. The cumulative heat stress index revealed that 2018 experienced the highest thermal stress values at both sites (Larvotto: 37.3; Portofino: 36.2), corresponding to extreme percentile ranks. In 2019, CHI values remained elevated, although lower, indicating persistent but less extreme thermal stress conditions.

(Continued on next page)

Figure 1. Cumulative heat index distribution based on the climatology in each site. The gray bar represents the range between the 10th and 90th percentiles for each site and year. Black dots and red triangles represent the median and target year’s value, respectively.



Discussion

Our results demonstrate that prolonged and cumulative warming, rather than short-term temperature increases, characterized the periods preceding mortality events. Thus, prolonged thermal stress appears to be a key factor in triggering NNV outbreaks. SST anomalies and CHI are effective environmental indicators for monitoring and forecasting disease risk. The high similarity between viral strains in wild and farmed fish suggests a shared epidemiological network, with potential bidirectional transmission. Climate change is expected to exacerbate these risks, increasing frequency of outbreak and duration. Integrating environmental indicators into aquaculture monitoring systems could improve risk assessment and management strategies.

Conclusions

Thermal anomalies and cumulative heat stress are key drivers of betanodavirus outbreaks. Integrated environmental monitoring systems are essential to support sustainable aquaculture under climate change.

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INTEGRATED SEASONAL EPIGENOMICS AND TRANSCRIPTOMICS UNCOVER AND VALIDATE NOVEL THERMAL-RESPONSIVE MARKERS IN GILTHEAD SEA BREAM

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Introduction

Global warming is rapidly reshaping marine ecosystems by increasing the frequency and intensity of thermal anomalies, thereby posing a major challenge to both wild and farmed fish in the Mediterranean basin, a recognised climate-change hotspot where warming rates exceed the global average (Atalah et al., 2024). Because temperature is a key driver of growth, metabolism, welfare, and disease resistance, Mediterranean aquaculture species such as gilthead sea bream (*Sparus aurata*) are particularly vulnerable to both seasonal variability and extreme thermal events. However, despite its importance, thermal tolerance remains a complex and multifactorial trait whose molecular basis is still poorly understood in most farmed fish (McCaw et al., 2020), especially with regard to the interplay between transcriptional and epigenetic regulation. In this context, integrative omics approaches have proven valuable for identifying robust welfare-related markers in gilthead sea bream (Belenguer et al., 2024), but their potential to elucidate the molecular mechanisms underlying seasonal thermal acclimation remains largely unexplored. To bridge this gap, we investigated a gilthead sea bream population that endured record marine heatwaves during the summer of 2022 while maintaining normal growth and production performance. Building on this, we integrated long-read epigenomic profiling with short-read transcriptomics across contrasting seasonal temperatures in metabolically active tissues. This approach enabled the identification of key molecular markers and regulatory mechanisms underlying thermal acclimation, which were subsequently validated in independent populations with the same genetic background under different warming and cooling scenarios.

Materials and methods

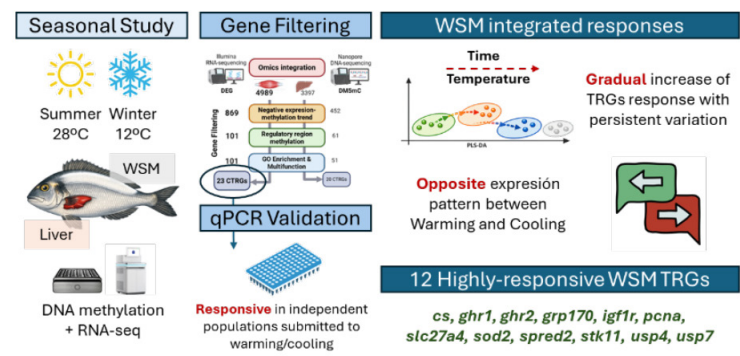
Gilthead sea bream from three production batches (2021, 2023, 2024) originating from the same Mediterranean hatchery (Avramar, Burriana, Spain) were reared from early life stages (~5 g) at the IATS experimental facilities. The first batch was assigned to the SEASON experiment, in which fish were maintained in 3000 L tanks from May 2022 to February 2023, with tissue sampling conducted in summer (July 2022; 28 °C; ~70 g) and winter (February 2023; 12.4 °C; ~340 g). Total DNA and RNA from white skeletal muscle (WSM) and liver were extracted and sequenced using Oxford Nanopore Technologies (PromethION 24) and Illumina NovaSeq 6000 platforms, respectively. DNA reads were basecalled with a modification-aware model, 5mC methylation (CG context) was identified using Modkit, and differentially methylated sites (DM5mC) were detected with MethylKit. RNA reads were mapped to the CSIC reference genome, and differentially expressed transcripts (DETs) were identified (FDR < 0.05). Integration of DETs with differentially methylated genes, combined with negative correlation analyses, regulatory-region filtering, and gene enrichment, enabled the identification of key molecular markers in both tissues. Additional filtering in WSM resulted in 23 candidate thermal-responsive genes (cTRGs), which were functionally validated as thermal responsive genes in separate trials, using fish from the 2023 and 2024 batches. In the WARMING-WINTER trial (February 2025), fish of ~360 g body weight were acclimated at 14 °C and then gradually exposed to 20 °C and 26 °C (~3 °C/day), with and intermediate sampling point. In the WARMING-SUMMER trial (June 2025), juvenile fish (~18 g) were exposed to either progressive warming (20–26 °C) or constant temperature (20 °C), followed by a common increase to 30 °C. In the COOLING-SUMMER trial (September 2025), fish (~184 g) acclimated at 28 °C were subjected to rapid cooling (~3 °C/day) to 22 °C and 16 °C, with sampling at each temperature step. Oxygen saturation remained above 75% in all trials.

Results and Discussion

Long-read ONT sequencing generated 825 Gb of data (avg. 29x genome coverage), detecting methylation at 78% of CG sites, with a prominent fraction found in gene bodies and promoter regions, confirming the suitability of the platform. Parallel RNA-seq identified 33,807 active transcripts. Seasonal comparisons revealed 6,356 DETs and 195k DM5mC in liver and 7,651 DETs and 302k DM5mC in the WSM. Omics integration and robust filtering highlighted 148 differentially expressed and methylated genes (DEMGs), as a reason of 51 in Liver and 101 in WSM (Fig. 1). Indeed, muscular responses

(Continued on next page)

Fig. 1. Graphical abstract of the main results in this study.



were higher at all layers and a diverse spectrum of functions, such as response to light, oxygen, and metabolic cues, were found as enriched. The 36 WSM cTRGs (23 from multi-omics and 13 extra genes) were further validated in the thermal-only trials. Of note, 31 out of 33 responded at least in one of the trials, becoming TRGs. PERMANOVA and PLSDA approaches showed a major temporal dynamic where the CTRL/initial sampling times were, regardless the trial, separated the most from the final points where temperature is maximum/minimum or has persisted. Once responsiveness and temporal dynamics were accredited for TRGs, our work further disclosed the major opposite expression change between WARMING and COOLING trials, mainly driven by up to 12 TRGs (*cs, ghr1, ghr2, grp170, igf1r, pcna, slc27a4, sod2, spread2, stk11, usp4, usp7*). These genes, involved in energetic, oxidative stress, cell proliferation and protein turnover cues, form an integrated molecular network that adjusts muscle physiology to thermal challenges. Altogether, this work opens novel perspectives to inform the effects of marine heatwaves in Mediterranean species and provides a mechanistic framework for understanding thermal resilience, supporting the development of genomic tools for climate-resilient aquaculture.

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REPRODUCTIVE VIABILITY OF WT/WT MALES AND SEX DETERMINATION IN FLATHEAD GREY MULLET (*Mugil Cephalus*), A STEP FORWARD

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Introduction

Mugil cephalus is a cosmopolitan species valued for its cured roes (*bottarga*). Its high market value drives the development of aquaculture practices, with the establishment of all-female populations being a key objective.

M. cephalus is gonochoristic species, lacking sexual dimorphism. Dor et al. (2016) identified a sex-determining locus suggesting an XY sex-determination system. Ferraresso et al. (2021) linked this to variants in the follicle-stimulating hormone receptor (*fshr*) gene, with females being homozygous (wt/wt) and males heterozygous (wt/m1 or wt/m2). However, homozygous (wt/wt) males occur in wild populations. Selecting such genotypes for breeding was proposed as strategy to produce female F1 progeny. Andree et al. (2024) validated a rapid, non-lethal, PCR-RFLP assay for genotyping, enabling early sex identification and broodstock selection. Authors used these tools to genotype male individuals in a captive F1 population showing skewed sex ratios (91.4% female), confirming that all tested males in that population were also homozygous wt/wt.

This study aimed to confirm if 1) wt/wt males can produce viable offspring and 2) the resulting progeny expresses the wt/wt genotype associated with a predominantly female phenotype.

Methods

In August 2025, a wild-caught wt/wt male identified by PCR-RFLP and confirmed by sequencing was selected based on sperm fluency and mated with a female (FAO Fisheries and Aquaculture Technical Papers No. 699). Fertilized eggs were transported to IMC laboratories, where rearing is ongoing. At 80dph animals were genotyped by both PCR-RFLP and sequencing. In October 2026 phenotypic sex of the offspring will be evaluated by histology to associate genotype with phenotype.

Results & Discussion

Progeny genotyping revealed 100% wt/wt genotype. Results of the genotype/phenotype association will be reported. This outcome contributes to a growing body of evidence that an XX/XY sex determination system in *Mugil cephalus* exists and demonstrates that wt/wt males can successfully be used to produce viable offspring with all female genotype.

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UPSTREAM MIGRATION OF THE INVASIVE BLUE CRAB IN THE PO RIVER, ITALY, HIGHLIGHTS THE VULNERABILITY OF FRESHWATER ECOSYSTEMS

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The Atlantic blue crab (*Callinectes sapidus*) is one of the most rapidly expanding invaders in the Mediterranean Sea, where it has established populations across all coastal and estuarine habitats. While its impacts in these environments are well documented, little is known about its occurrence and ecological role in inland waters. This study provides the first evidence of significant upstream migration of blue crabs along the Po River and nearby branches (Northern Italy), beyond typical coastal habitat distributions. Blue crab occurrences were based on 50 validated geo-referenced records collected between 2022 and 2025 through fishermen reports. Environmental variables and blue crab upstream distance were analyzed to explore possible drivers of this migration. The potential trophic interactions between blue crab and riverine fish species were also examined through a systematic literature review. The findings revealed that upstream dispersal reached distances of more than 160 km from the sea, primarily driven by adult males. No clear correlation was found between upstream distance reached by blue crab and water temperature, conductivity, dissolved oxygen, or river discharge, suggesting that ecological dynamics, rather than abiotic filtering, likely drive inland expansion. The literature review highlighted only a few documented trophic interactions in European freshwaters, although potential predation by invasive fish species and competition with native fauna may occur. These results underscore the urgent need for targeted monitoring and research to evaluate the ecological consequences of blue crab invasion in freshwater systems, particularly regarding its potential impacts on biodiversity, food web dynamics, and ecosystem functioning.

Acknowledgements

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BUILDING ENERGY MODELING OF RECIRCULATING AQUACULTURE SYSTEMS FOR DECARBONIZATION AND RENEWABLE ENERGY INTEGRATION

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Introduction

Global aquaculture is currently facing unprecedented challenges due to climate change, which threatens the stability of traditional aquatic ecosystems through rising temperatures, droughts, and extreme weather events (Ahmed et al. 2021). In this scenario of environmental vulnerability, recirculating aquaculture systems (RAS) emerge as a strategic and highly resilient solution (Badiola et al. 2018). However, this technological solution is accompanied by high energy requirements, mainly due to the use of water circulation pumps, aeration, filtration, temperature control using heat pumps, and indoor climate management (Martins et al. 2010). As a result, the environmental sustainability of RAS strongly depends on their energy performance and associated greenhouse gas emissions, making energy-efficient design and operation a key challenge for the sector (Dalsgaard et al. 2013). This study was developed in the framework of the Interreg Italy-Slovenia project “Circular Rainbow,” and the aim of this work is to develop and apply an integrated simulation model of a recirculating aquaculture system and its hosting building using TRNSYS (dynamic system simulation software used for building energy simulation) with the objective of quantifying energy consumption and evaluating the potential for emission reduction through energy efficiency measures and on-site renewable energy systems.

Materials and methods

The study evaluates the energy performance of an indoor pilot recirculating aquaculture system (RAS) through a dynamic simulation framework implemented in TRNSYS. The modeled system is a 900 m² facility with 5 hatchery tanks, 6 nursery tanks, 3 pre-growth tanks and 4 grow-out tanks, located in Udine, Italy. The estimated total production of the plant is 25000 kg of rainbow trout for a total water volume of 1000 m³ in the tanks. The system includes two sump loops, an equalization tank and two biofilter vessel loops. The TRNSYS simulation couples the model of the multizone building envelope and the water tanks model at an hourly time step, resolving transient heat and moisture exchanges (including water surface evaporation) driven by external weather data. Electrical loads for pumps, filtration and lighting are treated deterministically, while thermal demands are dynamically simulated. To evaluate efficiency and emission reduction strategies, three scenarios are compared against a baseline representing a reference system with conventional natural ventilation and building integrated photovoltaic (PV) panels:

Scenario 1: Integration of a photovoltaic (PV) system.

Scenario 2: Addition of battery storage for increased PV self-consumption.

Scenario 3: Implementation of optimized mechanical ventilation strategies.

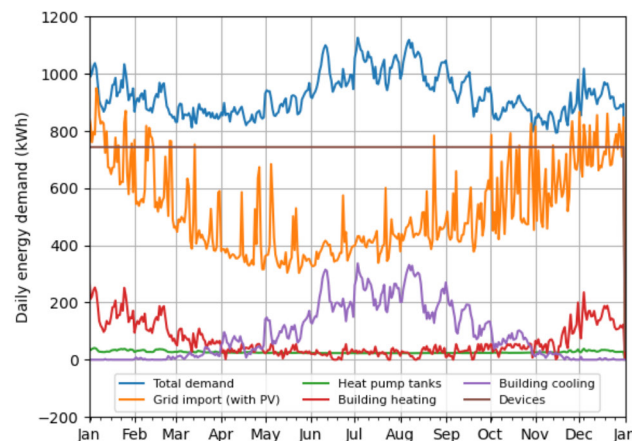


Figure 1: Daily energy demands by subsystem, total and net (Grid import (with PV)).

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Results & Discussion

The annual energy analysis of scenario 1 reveals that electricity demand to the grid can be reduced by the 44% with the building integrated photovoltaic on the roof surface reducing the energy demand from 14 kWh/kg of fish to 8 kWh/kg. These results are shown in Figure 1. The “total energy demand” is the sum of all the demands of the subsystems, while the “grid import (with PV)” indicates the net demand of energy including the energy produced by the photovoltaic panels. The “heat pump tanks” indicates the energy required to control the temperature of the water in the tanks, “devices” is the energy required by the circulating pumps and all the other devices, while the “building heating” and “building cooling” indicate the energy required to control the temperature and the humidity inside the building.

Scenario 2, including the battery storage system, allows to reduce the net energy demand to 1 kWh/kg of fish including a 100 kWh storage and an extension of 1000 m² of PV panels. The energy demand is used to estimate the carbon emissions and then are minimized by reducing the electricity demand from the grid with other combinations of PV and battery. Overall, the combined implementation of PV generation, battery storage, and ventilation strategies result in a significant reduction of annual carbon emissions compared to the baseline scenario. This study demonstrates the potential of integrated building and process simulation to support the design and operation of energy-efficient recirculating aquaculture systems. By coupling RAS tanks and building models in TRNSYS, it is possible to capture key interactions between water, air, and energy systems that strongly influence overall performance.

Funding

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GENETIC IMPROVEMENT OF POST-WINTER CONDITION INDEX IN YELLOWTAIL KINGFISH (*seriola lalandi*)

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Yellowtail Kingfish cultured in net pens can experience growth cessation and even weight loss during the winter period when temperatures fall below optimum. As an emerging, group spawning species, knowledge of genetic improvement strategies in this species is limited. Genetic improvement for winter resilience present an opportunity to improve productivity with shorter production cycles and increased eFCRs. This requires repeated individual weight measurements across a production cycle, which is impractical to evaluate on large net pens. Post winter condition index was evaluated across two generations of a yellowtail kingfish breeding programme representing six year classes. A moderate heritability of 0.28 was observed with an increase of post-winter condition index Breeding Values from 14.26 (± 0.03) to 14.43 (± 0.04) across generations. Genetic correlations of 0.47 are also shown between post-winter condition index and tank-based fasting daily growth coefficients (DGCs) corroborating supporting that post-winter assessment will enable selection for improved ability to maintain weight through winter. These results demonstrate the potential of selectively breeding for winter performance in yellowtail kingfish. In addition, it is one of the first examples of realized genetic gains from selective breeding in this species.

FUNCTIONAL FEED ADDITIVES REDUCE BACTERIAL-RELATED MORTALITY IN *Dicentrarchus labrax* ACROSS CONTROLLED AND FARM CONDITIONS

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Introduction

Bacterial infections remain a major constraint in Mediterranean farming of European sea bass (*Dicentrarchus labrax*), particularly during warm periods when disease pressure is highest. Pathogens such as *Photobacterium damsela* subsp. *piscicida* continue to cause significant mortality despite the use of vaccination and improved management strategies. In line with the sector's transition toward reduced use of antibiotics, functional feeds that support the fish's natural defence mechanisms are increasingly considered as preventive health tools.

Materials and Methods

The efficacy of a functional package composed of additives and microingredients was evaluated through two complementary experiments: a controlled bacterial challenge trial and a large-scale farm validation trial. The challenge trial consisted of a seven-week feeding period followed by a bath challenge with *Photobacterium damsela* subsp. *piscicida* under controlled conditions in juvenile sea bass (initial body weight 21.8 g) at 22°C. The field trial was conducted at a commercial sea bass grow-out site, with over 1.6 million fish (initial body weight 185 g), covering a natural high-risk period for bacterial outbreaks (August-November). Mortality was continuously monitored and relative percent survival (RPS) was calculated to compare fish fed a control diet with those receiving the functional additive package.

Results and Discussion

Under controlled conditions, fish fed the functional diet showed significantly improved survival following bacterial challenge, with mortality reduced by 11.9 % compared to the control group, corresponding to an approximate 32% improvement in RPS. Statistical analysis indicated a strong treatment effect, with a 98% probability of direction and an odds ratio of 1.75 whose uncertainty interval excludes 1.0, indicating a statistically significant association. These survival benefits were consistently confirmed under commercial farming conditions, where the functional diet reduced overall mortality during periods of elevated disease pressure. In the field trial, mortality was reduced by 1.69 %, resulting in a 47% improvement in RPS and a 97% probability of direction with an odds ratio of 1.91, corresponding to a 91% higher risk of mortality in the control group compared with the test group.

Conclusions

The combined evidence from controlled challenge and commercial field trials shows that functional feed additives can significantly reduce bacterial-related mortality in European sea bass. Such nutritional strategies represent a robust and sustainable complement to existing health management practices, supporting fish survival during predictable high-risk periods.

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DIETARY PHYTOGENICS IMPROVES SURVIVAL IN NILE TILAPIA (*Oreochromis niloticus*) COHABITATION CHALLENGE WITH *Streptococcus agalactiae*

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Introduction

Tilapia aquaculture worldwide is increasingly challenged by bacterial disease outbreaks, with streptococcosis representing one of the most relevant threats to production, especially under intensive farming conditions and elevated water temperatures. Streptococcal infections severely impair growth, feed efficiency, immune competence and survival, finally generating substantial economic losses and raising concerns about the sustainability of tilapia production. Among the nutritional strategies explored to support fish health and reduce disease susceptibility, dietary antimicrobial compounds, including botanical extracts, organic acids, and other bioactive feed additives, have shown potential to modulate gut microbiota, enhance innate immunity, and improve resilience against pathogenic bacteria (Kuebutornye e Abarike 2020; Radwan et al. 2022; de Aguiar et al. 2023; Liliana et al. 2024) and production globally was about 4.5 million tons in 2013 and is predicted to exceed 6.6 million tons by 2030. The emergence of diseases has led to significant loss to tilapia aquaculture over the years. Medicinal plants have been known for use in treating some human diseases and studied in aquaculture to find safe and eco-friendly compounds that are suitable alternatives to the use of antibiotics and chemical compounds. Several parts including seeds, roots, flowers, leaves, and their extracts have been applied in tilapia aquaculture, administered to fish through injection, immersion, and oral. The biological activities of medicinal plants include enhancement of growth and feed utilization, stimulation of the cellular and humoral immune response, gene expression, and increased disease resistance in tilapia. Despite the potential contribution of medicinal plants, there are some drawbacks including few commercially available medicinal products, no precise data on optimal dose requirements, lack of toxicity studies, and inadequate data on the effects of medicinal plants at the molecular level. This article describes the role of medicinal plants and their forms used to improve growth, feed utilization, immune response, and disease resistance as well as the prospects of medicinal plants in tilapia aquaculture.”,”container-title”:”Aquaculture International”,”DOI”:”10.1007/s10499-020-00506-3”,”ISSN”:”0967-6120, 1573-143X”,”issue”:”3”,”journalAbbreviation”:”Aquacult Int”,”language”:”en”,”page”:”965-983”,”source”:”DOI.org (Crossref. This study evaluated the impact of different doses of a microencapsulated phytogenic blend (PHYTO) on Nile tilapia (*Oreochromis niloticus*) subjected to a cohabitation challenge with *Streptococcus agalactiae*.

Materials and Methods

A total of 675 Nile tilapia (average weight 16.85 ± 0.2 g) were stocked into 9 tanks (670 L) within a freshwater recirculating aquaculture system and allocated randomly to 3 experimental groups (3 tanks/treatment, 75 fish/tank). Treated groups received dietary supplementation of PHYTO at 0.5 or 1 kg/MT of feed via top-coating (PHYTO 0.5 and PHYTO 1.0, respectively), while the control group (CTR) received unsupplemented feed. Growth phase lasted 28 days, then a 31 days cohabitation challenge was performed by transferring 20 fish from each experimental tank into a challenge unit with 8 fish inoculated with *S. agalactiae*. Fish were bulk-weighed on day 28 of growth phase and on day 31 post-challenge (dpc) to assess performance parameters while survival was monitored daily. Additionally, 5 fish per tank ($n = 15$ /treatment) were sacrificed to evaluate condition factor (K), hepatosomatic index (HSI) viscerosomatic index (VSI) and intestinal histopathological signs. Data were analyzed using one-way ANOVA with Tukey’s *post-hoc* test or Kruskal–Wallis test for non-parametric data, survival data were analysed using Kaplan–Meier analysis. Significance was set at $p \leq 0.05$, tendency was set at $0.05 \leq p \leq 0.1$ (GraphPad Prism v.11.0.1; GraphPad Software, Inc., Massachusetts, USA).

Results

Statistical analysis of performance did not reveal any significant difference in growth and challenge phase for any treatment. Survival analysis of the challenge phase highlighted a tendency for relative percent survival improvement of PHYTO 1.0 group compared to both PHYTO 0.5 and CTR groups (+29% RPS) with the last mortality event in the treated group recorded 9 days prior to CTR. Regarding body composition all the diets experienced similar K-index in both growth and challenge phase, however regarding VSI and HSI, only PHYTO 1.0 was able to maintain pre-challenge body composition indexes. Other groups instead experienced an increase compared to pre-challenge levels suggesting an ongoing hepatomegaly process during the cohabitation trial although with different degrees of severity (PHYTO 0.5 +20% HSI; CTR +51% HSI). From a histological perspective, treated groups showed numerical decrease in all pathological alterations evaluated after challenge compared to CTR with a significant decrease of erythrocyte extravasation in the intestinal lining and a statistical trend for the mitigation of cellular inflammatory infiltration for all supplemented groups.

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Conclusions

In conclusion, dietary supplementation with microencapsulated phytogetic compounds demonstrated potential as nutritional strategy to support Nile tilapia survival, resolve infection in shorter timing and finally mitigate clinical alterations during *S. agalactiae* challenge. These findings therefore support the integration of this phytogetic additive in tilapia feeds to promote innovative and sustainable intensive aquaculture nutrition practices. Moreover, further studies are necessary to optimize phytogetic dosages, application timing and finally confirm effectiveness against other aquatic pathogenic *Streptococcus* spp. strains.

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EARLY SYSTEMIC IMMUNE RESPONSE AND DISEASE RESISTANCE OF RAINBOW TROUT (*Oncorhynchus mykiss*) FOLLOWING COHABITATION CHALLENGE WITH *Yersinia ruckeri*

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Introduction

The limited preventive and therapeutic interventions permitted under organic aquaculture regulations increase the importance of timely disease diagnosis and effective health monitoring, not only to limit pathogen spread, reduce antibiotic use, and mitigate production losses, but also to establish humane intervention endpoints and reduce fish suffering (Beg et al., 2024; Lembo et al., 2019).

Yersiniosis, caused by *Yersinia ruckeri*, is one of the most important bacterial diseases affecting salmonids worldwide. However, the early host immune response to naturally transmitted infection remains poorly characterized, as experimental infection models commonly bypass the natural route of pathogen exposure, hindering the identification of reliable indicators of disease onset. To address this gap, a cohabitation challenge was established using gavage-inoculated shedder fish to characterize the early systemic response of recipient rainbow trout (*Oncorhynchus mykiss*).

Materials and Methods

Juvenile rainbow trout (~12 g) were randomly assigned to 120-L tanks (40 fish tank⁻¹). Following acclimation, 12 fish were sampled as uninfected controls (CTRL). Thereafter, 10 fish per tank were anesthetized and inoculated via oral gavage with *Y. ruckeri* strain QSP57.1 (3.5×10^{10} CFU mL⁻¹). The upper lobe of the caudal fin was clipped to identify orally inoculated fish, which were returned to their tanks as shedders, while the remaining 30 fish per tank served as naïve cohabitants (1:3 shedder-to-cohabitant ratio).

Nine shedder fish were sampled at 3, 6, 9, 24, 48, 72, 96 and 120 hours following oral gavage to quantify splenic bacterial load. Twelve cohabitant fish were sampled after 24 h of cohabitation (T0h), and subsequently at 3, 6, 24, 48, 72 and 96 hours (one tank per time point). In cohabitants, spleen samples were collected for bacterial load quantification, blood was collected for total red blood cell (RBC) and white blood cell (WBC) counts, hemoglobin concentration, mean corpuscular hemoglobin (MCH), and plasma humoral immune parameters. Head-kidney tissue was also collected at the indicated times for gene expression analysis. Three additional replicate tanks were used exclusively for daily mortality monitoring over 15 days.

Results and Discussion

Mortality was first observed in shedder fish at four days after oral gavage, while in cohabitant fish appeared one day later. After 15 days, cumulative mortality reached 53.3% and 34.4%, respectively (Figure 1). Probe-based qPCR confirmed that gavage-inoculated shedders successfully transmitted the pathogen to naïve cohabitants, with the prevalence of infected fish and splenic bacterial burden increasing over time in both groups.

Preliminary hematological analyses indicated that RBC counts remained stable throughout the study, whereas hemoglobin concentration, MCH and total WBC counts progressively declined, with significant reductions detected at 96 h. Ongoing analyses of plasma humoral immune parameters and gene expression will provide additional insight into the early systemic response to infection.

Conclusions

This study establishes oral-gavage-mediated cohabitation as a biologically relevant model for investigating the natural horizontal transmission of *Y. ruckeri* in rainbow trout, enabling the characterization of the early host responses and the future identification of early diagnostic biomarkers of infection.

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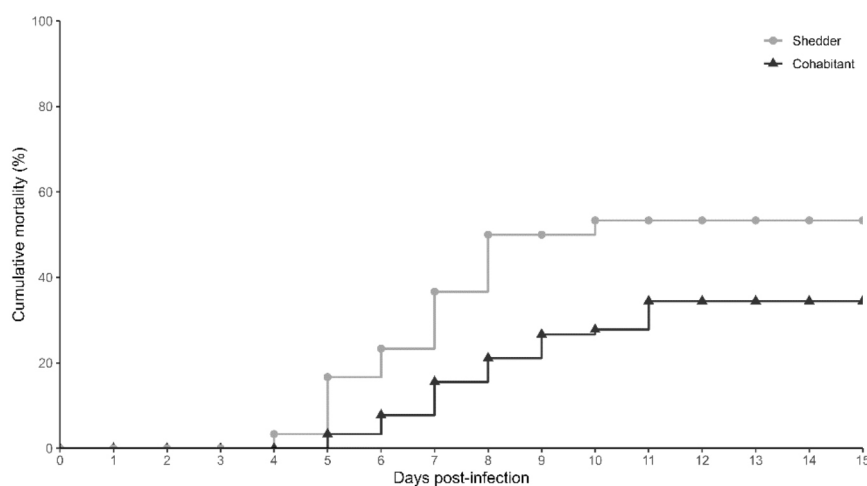


Figure 1. Cumulative mortality (%) of rainbow trout infected with *Y. ruckeri* by oral gavage (n = 30; ●) or cohabitation (n = 90; ▲).

Acknowledgements

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GROWTH PERFORMANCE OF *Macrocystis pyrifera* (LAMINARIALES) SPOROPHYTES CULTIVATED UNDER DIFFERENT SYSTEMS

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Introduction

The production of the macroalga *Macrocystis pyrifera* is mainly associated with the demand for alginate extraction (Vásquez et al., 2014), driving advances in cultivation techniques to improve its growth. The availability of nitrogen is essential for tissue formation, energy conversion, and storage in vacuoles and chloroplasts (Harrison and Hurd, 2001) productivity, epiphytes and the amount of certain products formed. Carbon, nitrogen and phosphorus are the main three nutrient elements added to large scale aquaculture systems. This review focuses mainly on nitrogen and the nitrogen physiology of seaweeds. Important concepts such as nutrient uptake, assimilation, storage, response to nutrient additions, nutrient ratios, and critical tissue nitrogen levels are discussed in terms of their application to aquaculture. For a particular species grown in an aquaculture system, it is important to know the C:N:P ratio of the seaweed under nutrient saturating conditions. This information will make the delivery of C, N, and P more economical. Nutrient uptake rates vary considerably with various physical, chemical and biological factors. In particular, light and temperature influence nutrient uptake rates, but we require further research to fully understand their effects. Nitrate uptake by some seaweeds is light dependent (i.e. diel periodicity). Rapid growth during early stages of production is essential for the viability and productivity of the culture. Consequently, the addition of commercial fertilizers to culture media can influence production costs. As an alternative to external inputs, Integrated Multitrophic Aquaculture (IMTA) recycles nutrients from the main species cultured into biomass of low-trophic-level organisms, such as macroalgae (Neori et al., 2004). Therefore, this study evaluated whether increasing nitrogen availability in a continuous-flow system with turbot (*Scophthalmus maximus*) affects the growth of two *M. pyrifera* morphotypes (few- and multiple-frond seedlings) in outdoor tanks and whether these effects persist after transfer to suspended longline culture at sea.

Materials and methods

In the winter of 2026, a two-part experiment was set. The first part lasted 21 days and consisted of two treatments with four replicates each: natural water (NW) and integrated aquaculture (F). The NW treatment used 1,000-L tanks with constant aeration and seawater recirculation, whereas the F treatment integrated 1,000-L macroalgae tanks with 1,500-L turbot tanks, with continuous water flow from the fish to the macroalgae tanks. Sixty juvenile *M. pyrifera* sporophytes per replicate, representing two morphotypes (few- and many-frond) (Buschmann et al., 2020), were used. Ten sporophytes of each morphotype were tagged for weekly weight monitoring. In the second-part experiment, the 20 tagged sporophytes from each tank were transferred to a longline at 1.5 m depth, and cultivated for 30 days. Forty sporophytes were collected and weighed after 15 days, and the remaining individuals after 30 days. Average weight gain (g) and SGR (% day⁻¹) were calculated. Water quality (ammonia, nitrite, nitrate, and phosphate) was monitored weekly during the experiment. Statistical analyses were performed using PAST v4.03. Data normality and homoscedasticity were assessed using the Shapiro–Wilk and Levene tests, respectively. One- or two-way ANOVA followed by Tukey's test was used when assumptions were met, with $p \leq 0.05$.

Table 1. Performance of *Macrocystis* juveniles in natural seawater (NW) and fish tank water (F) during the 21 days they were cultivated in land tanks and 30 days on a longline at sea.

	Land-based tanks (21 days)		Longline (30 days)	
	Seawater - NW	Fish tank - F	Seawater - NW	Fish tank - F
Few fronds				
Average weight gain (g)	3.81 ± 1.64	3.61 ± 1.24 B	11.26 ± 5.08 B ^b	26.76 ± 10.61 ^a
SGR (% day ⁻¹)	2.19 ± 1.26	2.49 ± 0.94	2.35 ± 1.05 ^b	4.43 ± 0.49 ^a
Many fronds				
Average weight gain (g)	5.21 ± 2.05	6.14 ± 2.13 A	37.00 ± 17.96 A	35.13 ± 7.34
SGR (% day ⁻¹)	1.86 ± 0.61	2.19 ± 1.08	3.92 ± 1.60	3.62 ± 0.72

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Results

Ammonia concentrations differed significantly between treatments on days 14 (NW 26.6 ± 1.7 b; F 34.0 ± 1.3 a $\mu\text{mol L}^{-1}$) and day 21 (NW 25.3 ± 1.7 b; F 31.8 ± 1.8 a $\mu\text{mol L}^{-1}$) in the tank culture. Nitrate concentrations differed significantly between treatments at the beginning of the cultivation (NW 13.2 ± 2.8 b; F 18.9 ± 1.8 a $\mu\text{mol L}^{-1}$). For phosphorus, significant differences were found on Day 0 (NW 2.4 ± 0.0 a; F 1.0 ± 0.5 b $\mu\text{mol L}^{-1}$) and on Day 21 (NW 2.3 ± 0.8 b; F 3.1 ± 0.1 a $\mu\text{mol L}^{-1}$). Weight gain did not differ significantly between treatments during the 21-day tank culture (Table 1). In contrast, during longline culture, the few-frond morphotype showed higher weight gain and specific growth rate in the F treatment than in the NW treatment (Table 1).

SGR specific growth rate. Different letters in the same line indicate a significant difference between treatments. Capital letters in the same column indicate a significant difference within the same treatment between groups of fronds.

Discussion

The higher ammonia and nitrate concentrations in treatment F resulted from animal excretion and feed leaching (Silva et al., 2013), compared to with seawater. During land-based cultivation, the higher growth of the “many fronds” morphotype in treatment F may indicate a competitive advantage resulting from enhanced light interception due to its higher number of fronds, potentially increasing shading and limiting light availability to the “few fronds” morphotype. The insignificant difference between treatments can be explained by space limitations as macroalgal biomass increased (Xiao et al., 2019). In contrast, under longline cultivation, the higher weight gain in treatment F may be associated with the higher nitrogen retention achieved during the tank cultivation phase, which may have promoted subsequent macroalgal growth (Harrison and Hurd, 2001). With the increased space available, the “few fronds” morphotype showed growth similar to that of the “many fronds” morphotype in the treatment F.

Acknowledgment

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THE GLASS-EEL-RESCUE PROJECT: AN INTEGRATED APPROACH TO SAFEGUARDING THE EUROPEAN EEL (*Anguilla anguilla*) THROUGH A RESTOCKING PROGRAM USING THE PCCR METHOD (POST-LARVAL CAPTURE, CULTURE AND RELEASE)

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Introduction

The European eel *Anguilla anguilla* is currently classified as critically endangered (Pike et al., 2020), mainly due to habitat loss, overfishing, migration barriers, environmental degradation and increasing predation pressure. The severe decline of both adult and juvenile stages has made the development of innovative conservation strategies a priority at European and regional levels (Dekker, 2003). Restocking with juvenile eels is considered one of the available tools to support stock recovery, although its effectiveness can be limited by high mortality during the early life stages after release (European Council, 2007). In this context, the Post-Larval Capture, Culture and Release approach may represent a useful strategy to improve survival by rearing glass eels under controlled conditions until they reach a more robust juvenile stage before restocking (Cortés-Useche et al., 2021). At the same time, the invasive blue crab *Callinectes sapidus* is rapidly expanding in Mediterranean coastal ecosystems, causing ecological and economic impacts (Marchessaux et al., 2023). The use of eggs from ovigerous blue crab females as a natural feed source for glass eels may provide a dual benefit: supporting eel growth and contributing to the management of an invasive species.

Materials and Methods

The project will be carried out at the aquaculture facilities of the University of Bologna, Cesenatico, Italy. Glass eels will be collected during their natural migration in transitional waters and river mouths of Emilia-Romagna, or, if necessary, obtained from certified suppliers. After transport to the research facility, animals will be acclimated to controlled rearing conditions and a representative sample will be measured for body weight, total length and Fulton's condition factor. Eels will be maintained in recirculating aquaculture systems equipped with mechanical and biological filtration, temperature and oxygen control, UV and ozone water treatment. During the rearing phase, different feeding protocols based on eggs of *C. sapidus* will be tested and compared in terms of growth performance, survival, health status and welfare indicators. Water quality parameters, including temperature, dissolved oxygen, salinity, pH and nitrogen compounds, will be monitored daily. At the end of the culture period, juveniles will undergo biometric and health assessment and will be gradually acclimated to the environmental conditions of selected release sites.

Results

The expected outcome of the project is the development of a sustainable rearing protocol for glass eels aimed at improving survival and growth before release. Preliminary observations conducted by the research group suggest that diets including blue crab eggs may be suitable for the nutritional requirements of glass eels and may support very high survival rates under controlled conditions. The controlled rearing phase is expected to reduce early mortality by limiting predation, environmental stress and feeding difficulties, allowing the release of larger and more robust juveniles. The project will also generate data on the suitability of *C. sapidus* eggs as a natural feed ingredient, with particular attention to growth response, animal condition and practical applicability in conservation-oriented aquaculture.

Discussion

This approach integrates eel conservation, sustainable aquaculture and invasive species management within a single applied framework. Rearing glass eels to a more resistant juvenile stage before release could improve the effectiveness of restocking programmes, especially in areas where natural recruitment is low and early-stage mortality is high (Richardson et al., 2023). The use of blue crab eggs as feed may reduce reliance on commercial inert diets and provide a more natural and sensory-stimulating food source for juvenile eels. In addition, the removal of ovigerous blue crab females could contribute, although locally and partially, to reducing the reproductive pressure of this invasive species (Marchessaux et al., 2023). If successful, this pilot protocol could be replicated at regional and national levels, supporting conservation actions for *A. anguilla* while promoting circular and ecosystem-based solutions in aquaculture.

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MONITORING OF *Photobacterium damsela* subsp. *piscicida* INFECTION IN SEA BASS (*Dicentrarchus labrax*) USING THREE FUNCTIONAL DETECTION METHODS

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Introduction

Fish pasteurellosis or photobacteriosis caused by *Photobacterium damsela* subsp. *piscicida* (*Pdp*) is one of the most significant bacterial diseases affecting marine aquaculture in Europe, particularly in European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) (EU Reference Laboratory for Fish and Crustacean Diseases, 2021). Despite its significant economic impact, the detection and diagnosis of this pathogen remain challenging. Conventional microbiological isolation is hindered by its inability to grow on selective media such as TCBS agar, its slow growth rate, and its lack of distinctive colony morphology. Molecular detection is also limited by the absence of subspecies-specific PCR primers, as currently available assays generally target the species level and cannot reliably discriminate *P. damsela* subsp. *piscicida* from the closely related *P. damsela* subsp. *damsela* (Andreoni & Magnani, 2014). Multiplex PCR assays based on 16S rRNA gene detection and the absence of *ureC* amplification have been developed as an improved approach for discriminating the pathogen at the subspecies level (Osorio et al., 2000). However, PCR-based methods have certain limitations when assessing bacterial pathogenicity, as they primarily provide information on bacterial abundance rather than direct evidence of virulence or pathogenic potential within the detected population.

To address these limitations and improve our understanding of the pathogen's infection dynamics, an in vivo challenge was conducted at CTAQUA (Centro Tecnológico de la Acuicultura, Cádiz, Spain) in which European sea bass (*Dicentrarchus labrax*) were experimentally infected with *Pdp*. The study aimed to monitor disease progression and evaluate pathogen detection in the aquatic environment throughout the course of an infection using microbiological techniques, phenotypic virulence assays, and a set of experimental biosensors developed by KOA Biotech.

Materials and Methods

Two experimental groups of European sea bass, were challenged by immersion with *Pdp* at final concentrations of 1.65×10^7 CFU/mL and 1.65×10^4 CFU/mL, respectively. Each experimental batch included three control tanks and three infected tanks. Water quality parameters were monitored daily, and fish mortalities were recorded throughout the challenge.

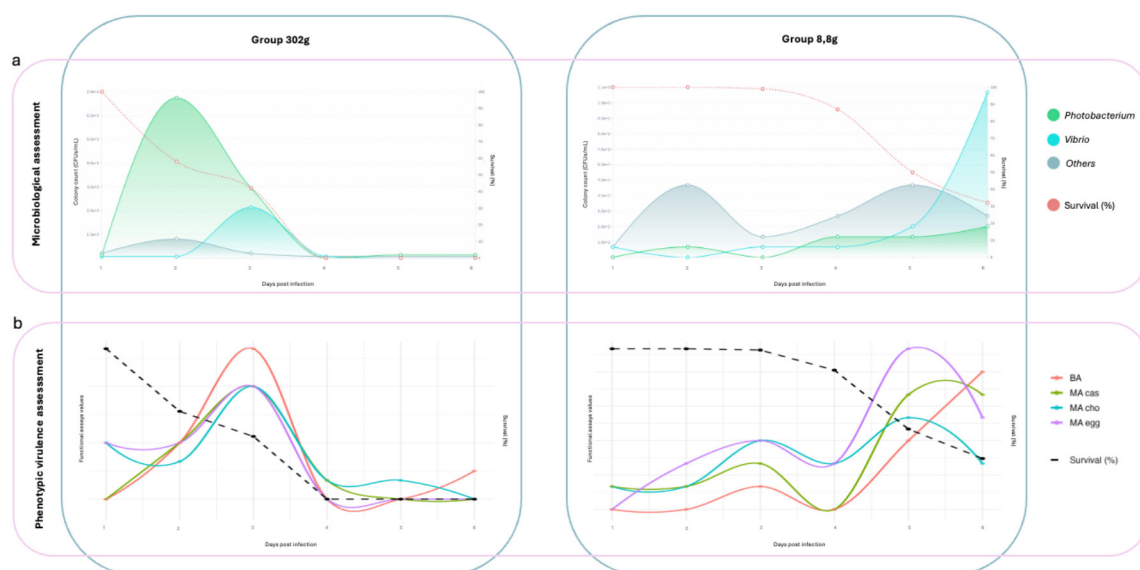


Figure 1. Comparison of microbiological and phenotypic Virulence Assessment methods for both fish groups. The x-axis shows the days post-challenge, while the y-axis displays bacterial load in CFU/mL (a) and functional assay activity (b).

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Water samples were collected daily for pathogen growth assessment. Samples were cultured on TCBS, TSA supplemented with 2% NaCl, and blood agar, with bacterial colonies quantified using an automated detection algorithm. Phenotypic virulence was assessed using four functional media targeting chondroitin sulfate, egg yolk, casein, and blood degradation. Pathogen levels were also monitored using KOA Biotech's experimental biosensors.

To verify the infection caused by *Pdp*, all deceased fish underwent external and internal necropsy, followed by bacteriological isolation from the head kidney. Pure cultures obtained on TSA supplemented with 2% NaCl were subsequently streaked onto TCBS agar to confirm the absence of bacterial growth on this selective medium.

Results

At the microbiological level, three bacterial populations were monitored: *Photobacterium damsela* subsp. *piscicida*., *Vibrio* spp., and “Other” bacteria, the latter classified as potentially protective bacteria identified within the water microbiome. The results showed peaks in *Photobacterium* CFU/mL at time points coinciding with increases in fish mortality. An antagonistic dynamic was also observed between the *Photobacterium* and *Vibrio* populations, together with an overall decline in the “Other” bacterial population.

At the functional level, the observed peaks correlated with the first mortalities, supporting the high pathogenic activity of the pathogen and the high mortality rates reached during the challenge.

Finally, at the biosensor level, an increase in the biosensor response was detected several days before the onset of the first mortalities.

Conclusions

Collectively, these results demonstrate that a multimethodological approach provides a more comprehensive understanding of the infection dynamics of *Photobacterium damsela* subsp. *piscicida*, and may contribute to improving its diagnosis in aquaculture facilities. The methodologies employed focus primarily on the functional characterization of pathogen presence rather than on genomic detection alone, potentially providing a more comprehensive understanding of the virulence dynamics that may develop during infection, as well as of the microbiological status of the surrounding water.

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RESEARCH INTO THE CULTIVATION POTENTIAL OF THE EUROPEAN FLAT OYSTER IN THE ADRIATIC SEA

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Summary

The aim of the MARINET project (italy-croatia.eu/web/marinet) is to investigate the potential for cultivating the European flat oyster (*Ostrea edulis*) at new potential sites in the Adriatic Sea and to expand its cultivation. The assessment of cultivation potential is based on the environmental conditions under which the European flat oyster can grow and the potential for spat settlement. For this purpose, a comparative analysis was conducted of sites characterised by naturally elevated phytoplankton abundance and the presence of a natural oyster population in the vicinity. Based on these criteria, nine sites were selected: four in Istria (Raša Bay, Budava Bay, Lim Bay and Savudrija) and five along the Italian Adriatic coast (Muggia, Duino, Cattolica, Mattinata and Gallipoli).

At each site, European flat oyster specimens were deployed to monitor their growth and mortality, as well as the environmental conditions under which they developed. In addition, collectors for oyster spat settlement were deployed at the sites in Istria and at Muggia, Duino and Cattolica. At sites where depths exceeded 10 metres, additional oyster specimens and collectors were deployed at greater depths to investigate the effects of depth and temperature on growth, spat settlement and the degree of fouling. To ensure that the results were as representative as possible of future production conditions, oyster sampling was conducted every two months, following a maintenance regime similar to that which would be applied under actual production conditions.

Growth rates were generally similar among the sites, except at Gallipoli and Mattinata, where the lowest growth rates were recorded. In contrast, the highest growth rates were observed at the northern Adriatic sites, namely Muggia, Lim, Savudrija, Duino, Raša, Budava and Cattolica. Regarding mortality, the European flat oyster showed a similar pattern across the sites, with higher mortality observed during periods of summer heat and lower mortality during the remainder of the year. Differences among the sites indicated that the lowest mortality rates were again observed at Muggia, Savudrija, Lim, Raša and Budava. Furthermore, with regard to spat recruitment potential, only Muggia and Savudrija showed higher recruitment levels, followed by moderate recruitment at Lim and Budava. This may be explained by the proximity of these sites to local oyster populations and the transport of sufficient numbers of larvae by marine currents to the collectors.

At the sites of Budava, Duino, Mattinata and Gallipoli, an additional analysis was conducted to determine whether the application of Integrated Multitrophic Aquaculture (IMTA) provided any benefits. The results indicated that the flat oysters at all sites ingested and utilised by-products of fish aquaculture; however, no noticeable increase in growth rates was observed compared with control samples at the same sites.

We conclude that each site has its own specific characteristics, strengths and weaknesses, and that all these aspects should be taken into consideration when planning future flat oyster aquaculture production sites. Overall, the most promising sites appear to be Muggia, Savudrija, Budava and Lim. However, coordinated production across all sites could maximise the aquaculture potential of the European flat oyster in the Adriatic Sea. A key aspect will be to adapt future aquaculture practices to local environmental and climatic conditions. It is therefore also recommended that environmental conditions continue to be monitored and that forecasting tools be developed to assess future risks and serve as early warning systems for aquaculture.

EVALUATION OF THE EFFECTS OF DIFFERENT CONCENTRATIONS OF TWO GRAPE-DERIVED POLYPHENOL-RICH ADDITIVES ON RAINBOW TROUT (*Oncorhynchus mykiss*) UNDER THERMAL STRESS CONDITIONS

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Introduction

The rapid expansion of the aquaculture industry, combined with the ongoing effects of climate change, has increased the exposure of farmed fish to environmental stressors, among which temperature fluctuations represent one of the most critical challenges. Elevated water temperatures can disrupt metabolic balance, impair immune function, and promote the overproduction of reactive oxygen species (ROS), leading to oxidative stress and cellular damage.

In this context, dietary strategies based on natural compounds have gained increasing attention. Polyphenols, a diverse class of plant-derived bioactive molecules, are known for their strong antioxidant and anti-inflammatory properties and their ability to modulate immune responses. Grape by-products from the wine industry represent an important and sustainable source of such polyphenols, offering a valuable opportunity for the valorisation of agro-industrial waste through their conversion into functional feed ingredients for aquaculture (Quagliardi et al. 2024).

The inclusion of polyphenol-rich additives in aquafeeds has been associated with improved growth performance, enhanced antioxidant capacity, and increased resistance to stress in several fish species (Mansoori et al. 2024; Ahmadifar et al. 2012; Torricelli et al. 2024). However, the efficacy of different polyphenol sources and their optimal dietary concentrations, particularly under thermal stress conditions, remains to be fully elucidated.

Therefore, the present study aimed to evaluate the effects of different concentrations of two grape-derived polyphenol-based additives on growth performance, oxidative status, and selected biochemical parameters in rainbow trout under thermal stress conditions.

Materials and Methods

Five experimental diets were formulated: four supplemented with two polyphenol-based additives at two inclusion levels, and a control diet without additives.

A total of 450 rainbow trout (*Oncorhynchus mykiss*) (initial weight: 30 g) were randomly distributed into experimental groups and fed for 60 days. Fish were hand-fed to apparent satiation twice daily, and water temperature (13.0 ± 1 °C) and oxygen levels were continuously monitored.

At the end of the trial, growth performance (FBW, SGR, FI, FCR, survival) and morphometric and nutrient efficiency indices (CF, VSI, HSI, MFI, PER, GPR, LER, GLE) were evaluated. Samples were collected to assess carcass composition, liver histology, gene expression of inflammatory markers, plasma biochemistry, liver metabolomics and oxidative stress biomarkers (total antioxidant capacity (TAC), glutathione (GSH), antioxidant enzyme activity (CAT), lipid peroxidation (TBARS), protein carbonylation (PC), and mitochondrial ROS production (mtROS)).

Fish were then subjected to a thermal stress challenge, with temperature increased to 20 °C (2°C day⁻¹) and maintained for 15 days. At the end of the stress period, survival and growth parameters were recorded, and sampling was repeated to assess physiological and biochemical responses.

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Results and Discussion

Fish fed diets supplemented with polyphenol-based additives are expected to show growth performance, FI, and FCR comparable to or slightly improved compared to the control group, particularly at moderate inclusion levels. Recent studies have reported enhanced growth and feed utilization in rainbow trout fed polyphenol-rich diets, likely due to improved metabolic efficiency and antioxidant status (Mansoori et al. 2024).

Oxidative stress biomarkers are expected to be significantly affected by dietary treatments. Fish receiving polyphenol-supplemented diets are likely to exhibit higher TAC, increased GSH, and enhanced CAT activity, along with reduced lipid peroxidation, protein carbonylation, and mitochondrial ROS production. These responses are consistent with findings highlighting the antioxidant role of polyphenols in fish (Ahmadifar et al. 2012).

Gene expression analyses are expected to show modulation of immune and inflammatory markers, with a potential reduction of pro-inflammatory cytokines and improved immune status, as recently observed in fish fed plant-derived bioactive compounds (Torricelli et al. 2024).

Histological observations are likely to reveal generally preserved intestinal and hepatic structures, with possible improvements at moderate inclusion levels and mild alterations at higher doses, reflecting metabolic adaptation.

Following the thermal stress challenge, fish are expected to show increased oxidative stress and physiological disturbance due to elevated temperature, as widely reported for rainbow trout (Hosseinpour et al. 2024; Yousefi et al. 2024). However, fish fed polyphenol-enriched diets are likely to exhibit improved resilience, with higher survival rates and better maintenance of antioxidant defences.

Overall, polyphenol supplementation is expected to enhance antioxidant capacity and improve the resilience of rainbow trout under thermal stress conditions.

Conclusion

Overall, the trial is expected to show that moderate inclusion levels of polyphenol-based additives can enhance antioxidant status and support immune responses in rainbow trout without negatively affecting growth performance. These effects are likely to be more evident under thermal stress, improving fish resilience and reducing oxidative damage.

Given the impact of climate change on aquaculture, particularly rising water temperatures, polyphenols represent a promising strategy to improve fish robustness and support more sustainable production systems.

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BIOECONOMIC MODELS IN MARICULTURE: A FOCUS ON FLAT OYSTER (*Ostrea edulis*) PRODUCTION OPPORTUNITIES IN THE ADRIATIC SEA

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Introduction

European flat oyster (*Ostrea edulis*) is a bivalve mollusc belonging to the family *Ostreidae*, a native European species that has inhabited the Mediterranean basin since prehistoric times. Although this organism has been a commercially important resource in Europe for centuries, its population has undergone a substantial decline in most European countries as a consequence of disease emergence, environmental pollution, and climate-change-related stressors (Brundu et al. 2025). Given the importance of promoting biodiversity and preserving the European flat oyster, this species is included as a focus of the 'Marinet' Interreg project (italy-croatia.eu/web/marinet). The project is promoting and developing a sustainable aquaculture in the Adriatic context, especially by empowering small and medium-sized enterprises (SMEs). One of the project's goals is the development of useful decisional support tools, such as bioeconomic models, mathematical tools capable of estimating the profitability of an organism production process (Garza, 2024). Based on these premises, this study proposes a bioeconomic model to assess the economic feasibility of European flat oyster production in several case study sites in the Adriatic Sea.

Bioeconomic Model Framework

Bioeconomic model approaches consist in a composition of interacting mathematical sub-models, developed using adapted procedures according to Chary et al. (2022). In aquaculture context, a framework of three interconnected sub-models (biological, technological, and economic) is used (Garza, 2024). The biological sub-model predicts the growth and survival rate of the farmed organism, determining the final product biomass and consequently the possible revenues. The biological sub-model comprises three sections: spat collection, growth, and population dynamics. Growth and population models estimate individual monthly growth and biomass dynamics based on initial stock size and survival rates (Kamiyama et al., 2021). The technological sub-model estimates the farm's technological operations energy requirements. Considering the off-shore aquaculture context, the energy requirements are represented by fuel consumption needed to carry on working operations, such as maintenance of long line and oyster husbandry activities and by the electrical energy required to operate the side activities in the oyster farm. Finally, the last sub-model is the economical one, where results from biological and technological sub-models are combined with external economic data to key economic performance indicators of the farm (KPI). This sub-model is divided in yearly fixed and variable cost estimation, revenue estimation and final KPI indicators calculation, focusing in particular on break-even quantity (*BEP Q*) which represents the requested quantity of production to cover all the production costs (Jolly e Clonts, 1993).

Data Implementation and Results

To generate projections the model needs to be fed with environmental and in situ specific observed biological measurement data, in order to mimic possible local practices and growth conditions. The model is used to generate KPI taking in consideration a hypothetical offshore long line farm of flat oysters situated in the Adriatic Sea. Biological data were recorded in nine different farms involved in the project, located in Croatia and Italy, and were used to produce weight growth and survival models. To estimate fuel consumption in the technological submodel we use as raw data monthly operational hours of the boats and hourly fuel consumption ratio, obtaining the fuel volume of fuel utilized. However, they were selected to ensure that overall fuel consumption remained consistent with the values reported in oyster farm life cycle assessment studies (Summa et al., 2023). Regarding the economic sub-model, fixed costs were estimated using a rough comprehensive approach. Variable costs, on the other hand, were calculated by multiplying the monthly fuel consumption by an assumed fuel price. Among variable costs we find also monthly staff and utility costs. Revenues are estimated monthly, considering that the farmer will sell a certain percentage of the available product, which can vary on the basis of market demand and proposed price. Selling prices were estimated BMTI monthly market sale data (bmti.it/analisi-ittico/). The final output of the model are KPI, such as BEP, cumulative cost and revenues graphs or yearly profits evaluations. These support decision tools consent to evaluate different farm management strategic decisions. In fact, the results of this study provide an assessment of the differences in economic opportunities among the various production sites, and comparison among different scenarios obtained by varying systematically different parameters, such as selling prices or technological parameters.

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Conclusion

The outputs of the bio-economic model provide useful indicators to support farm managers in strategy decisions. At the same time, the model offers a structured framework for collecting, organizing, and analyzing farm data, thereby facilitating the monitoring of farm performance and operations. As this bio-economic model represents a first approach, its current structure should be considered flexible and adaptable. The proposed sub-models can be refined and modified according to site-specific conditions, data availability, and farmers' needs.

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UNVEILING THE IMPACT OF SYNTHETIC VS. BIO-BASED MICROPLASTICS ON FARMED FISH ANTIOXIDANT DEFENSES AND DIGESTIVE HEALTH

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Introduction

Over the past decades, plastic pollution is a growing environmental concern, particularly in coastal and estuarine ecosystems where aquaculture production often takes place. Farmed species, especially those raised in extensive and semi-intensive systems, face increasing exposure to environmental contaminants, including plastic debris, discarded fishing gear, and other forms of marine litter. This exposure can pose risks to aquaculture industry through ingestion and the release of harmful chemicals into the surrounding water.

These concerns have intensified efforts to find alternatives to conventional plastics derived from fossil fuels. Among current alternatives, bioplastics have gained attention as more sustainable materials. The plastic industry often presents these polymers as environmentally friendly due to origin from renewable biological sources and, in some cases, greater biodegradation potential. Yet this same biodegradation potential raises ecological concerns, since bio-based polymers might fragment into microplastics more quickly than conventional petroleum-based plastics and release additives.

Current knowledge about the ecotoxicological effects of bioplastics remains limited, especially for recently developed polymers. This lack of information restricts an accurate assessment of the real environmental safety of bio-based plastics. Given the vulnerability of aquaculture species to marine litter and emerging plastic alternatives, further research is needed to assess biological impacts of these materials.

The present study aimed to investigate the ecotoxicological responses of farmed juvenile gilthead seabream (*Sparus aurata*) exposed to microparticles from three bio-based polymers: PLA (polylactic acid microplastics), PPF (polypropylene 2,5-furandicarboxylate microplastics), or a mix of PLA/PPF microplastics. Animal condition, metabolism, digestive and antioxidant activity, and cell damage to evaluate potential physiological effects associated with chronic exposure to these emerging materials were assessed.

Materials and Methods

Farmed *S. aurata* juveniles (18.78 ± 0.51 cm total length, 103.46 ± 5.52 g weight) were kept in recirculating aquaculture systems (RAS) under optimal rearing conditions: 24.0 ± 0.5 °C; (ii) dissolved oxygen: 7.2 ± 0.2 mg L⁻¹; (iii) salinity: 35.0 ± 0.5 ‰; (iv) pH: 8.0 ± 0.1 ; and (v) photoperiod: 12 h light/12 h dark.. During the 28-day exposure trial, specimens were fed 1% body weight of either a commercial-based diet (CTR) or one of three microplastic-contaminated diet, containing either i) PLA; ii) PPF, or iii) mixed polymers PLA/PPF microplastics. The three contaminated feeds contained ~60 particles g⁻¹ feed, each ranging between 500 and 1000 µm.

After 28 days of exposure trial, fish (n = 7 per treatment) were randomly collected and euthanized for dissection and collection of samples. To evaluate the ecotoxicological effects elicited by biobased microplastics, the following endpoints were assessed: animal condition, plasma metabolites, gut and liver biomarker responses indicative of oxidative stress (catalase, glutathione S-transferase, and superoxide dismutase activities), cell damage (ubiquitin content and lipid peroxidation), and digestive enzymes (trypsin, pepsins and amylase). Statistical differences between treatments were analyzed through one-way ANOVA.

Results and Discussion

Preliminary results show no significant alterations in fish condition between treatments, but significant differences in plasma metabolite profiles were registered. The most notable changes were found in glucose levels (+118%) of fish from the PLA+PPF treatment, and the total protein content (-16.7%) in fish from the PLA treatment.

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Regarding oxidative stress responses, in comparison with the control treatment, exposure to all biopolymers led to a reduction in the activity of antioxidant and detoxification enzymes present in fish gut. Still, there was no significant lipid damage observed in these fish.

Although data analysis is still ongoing, the results gathered so far suggest that while the tested materials did not affect fish condition, they may induce distinct metabolic responses, highlighting biological significance and potential long-term implications of biopolymers exposure in farmed juvenile fish. Hence, further research should be carried on bio-based polymers' ecotoxicological attributes, as to better support the “green” claims intended by these materials.

Acknowledgment

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INVASIVE–NATIVE FISH DYNAMICS IN A CHANGING CLIMATE: A MULTI-FACETED APPROACH FOR NATIVE FISH CONSERVATION IN A PENINSULAR RIVER NETWORK

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Introduction

Freshwater ecosystems possess high biodiversity supporting approximately 10% of all the species while occupying less than 1% of earth surface. In India freshwater ecosystem faces multiple anthropogenic pressures, including habitat degradation, pollution, flow modification, overexploitation, with climate change emerging as a widespread driver of ecological change. These stressors initiate conditions that favour the establishment and spread of invasive species while simultaneously affecting the resilience of native fish communities. Freshwater fish species are highly sensitive to temperature and especially vulnerable to climate-driven environmental changes. The impacts of climate change on fish distributions are projected to be severe, with studies predicting significant range contractions and shifts in freshwater communities.

Material and Methods

This current research aims to assess the habitat suitability of invasive-native under various environmental factors and highlight the implications for conservation strategies. Understanding water quality impacts and MaxEnt modelling, we evaluated the influence of spatial and environmental factors on native-invasive assemblages to determine invasion and environmental factors contributed to major alterations in native distribution pattern and predicting potential habitat suitability of invasive-native under climatic scenarios SSP1-2.6, SSP2-4.5, and SSP5-8.5 for the years 2050 and 2070. The species occurrence was documented in the Arkavathy River watershed, situated in a peninsular region was chosen for the study due to damming sites and angling reserve locations such as the Cauvery Wildlife Sanctuary. A total of 50 sampling points were chosen along the streams, covering the stretch from confluence to origin, with elevation ranging from 891 to 396 m above sea level.

Results

Redundancy analysis showed 82.24% explained variance, therefore, this model can help in future prediction of the effects of invasive alien species on native species assemblages. The MaxEnt model demonstrated high predictive accuracy, identifying 123.06 km² out of 4135 km² of highly suitable native species habitat, with future projections indicating a significant decline in suitable habitats by the year 2050 and 2070. Key environmental variables influencing habitat suitability included maximum temperature of warmest month, temperature seasonality, and isothermality.

Discussion

This study indicates that water quality monitoring is a good indicator of understanding shifts in the invasive-native distributional patterns. These findings underscore the urgent need for targeted conservation strategies to mitigate the impacts of climate change and anthropogenic pressures on endemic species, providing critical insights for the initial stages of developing policies for environmental restoration and fishery management.

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INTEGRATING LOW-TROPHIC AQUACULTURE WITH OFFSHORE WIND FARMS: ENVIRONMENTAL AND ECONOMIC PERFORMANCE OF MULTI-USE SYSTEMS

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Introduction

The continued expansion of offshore renewable energy infrastructure creates new opportunities for multi-use marine systems, in which low-trophic aquaculture can be co-located with offshore wind farms and potentially share spatial, infrastructural, and operational resources. Such integration may increase the efficiency of marine space use, reduce resource requirements and environmental burdens, and improve the economic feasibility of offshore aquaculture. This study evaluated the environmental and economic performance of offshore cultivation of sugar kelp (*Saccharina latissima*) and blue mussel (*Mytilus edulis*) as mono- and co-cultivation systems integrated with offshore wind farm operations under Danish offshore conditions. The analysis considered a commercial-scale cultivation area of 18.75 ha and investigated whether operational synergies associated with multi-use offshore systems could improve performance relative to standalone aquaculture.

Methodology

An integrated life cycle assessment (LCA) and techno-economic analysis (TEA) framework was applied to mussel mono-cultivation, seaweed mono-cultivation, and seaweed–mussel co-cultivation. Wind farm integration was modelled through shared offshore operations, including vessel use, diesel consumption, and monitoring activities. Environmental performance was evaluated using the Environmental Footprint (EF) 3.1 method. A separate scenario accounting for nutrient assimilation was evaluated to distinguish production-related impacts from potential ecosystem services benefits associated with nutrient removal. Economic feasibility was assessed over a 10-year project lifetime using Net Present Value (NPV), considering alternative market pathways for food- and feed-grade biomass.

Results and Discussion

Integration with offshore wind farm operations improved environmental performance across the cultivation systems. At farm scale, shared resource use reduced the environmental footprint of the co-cultivation system by approximately 17% relative to standalone cultivation. The carbon footprint decreased by approximately 4% for mussel mono-cultivation and 12% for seaweed mono-cultivation following wind farm integration, demonstrating that sharing infrastructure and offshore operations can reduce burdens associated with offshore logistics and farm management.

Economic viability was strongly influenced by both wind farm integration and the targeted biomass market. The highest economic benefit was obtained for co-cultivation combining high-value seaweed products with food-grade mussels, for which the 10-year NPV increased from approximately €10.8 million for standalone cultivation to €12.5 million following wind farm integration. In contrast, systems dependent on wholesale seaweed and feed-grade mussel markets remained economically constrained, indicating that operational synergies alone may not compensate for low biomass market values.

Conclusion

The combined environmental and economic assessment demonstrates that integrating low-trophic aquaculture with offshore wind farms can improve environmental and economic performance, particularly when shared infrastructure and operations are coupled with higher-value biomass applications. Co-cultivation showed the strongest overall benefit, supporting multi-use offshore systems as a pathway towards more resource-efficient and economically resilient marine production. However, market positioning remains a critical determinant of economic viability, highlighting the importance of integrating production-system design with biomass valorisation strategies.

Acknowledgement

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CIFARM, A NEW OPPORTUNITY TO INCREASE DOMESTIC SEAFOOD PRODUCTION IN US FEDERAL WATERS AND EXCLUSIVE ECONOMIC ZONE

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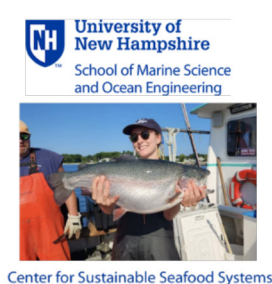
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The new Cooperative Institute Fostering Aquaculture Research and Marketing (**CIFARM**) was recently established in the US to expand seafood production and markets throughout the U.S. by developing aquaculture in territorial seas and the Exclusive Economic Zone, thereby enhancing coastal economies and supporting American maritime communities. The University of New Hampshire (UNH) was chosen as the lead institution by the National Oceanic Atmospheric Administration (NOAA) Office of Aquaculture (OAQ), along with Consortium members the University of Miami, University of Southern Mississippi, Hubbs SeaWorld Research Institute, and the University of Hawai'i, with regional Sea Grant Programs. CIFARM will work closely with state, federal and industry partners to foster sustainable aquaculture across diverse U.S. regions by establishing a coordinated framework that leverages regional expertise, streamlines permitting processes, and promotes innovation and workforce development.

This initiative aligns with the NOAA Aquaculture Strategic Plan goals, which include managing sustainability and efficiency, leading with science, educating and exchanging information, and supporting economic viability and growth.

Each CIFARM Consortium member, strategically located across the Northeast, Southeast and Caribbean, Gulf of America, West Coast, and the Pacific Islands, is designed to embody excellence in state-of-the-art infrastructure, aquaculture science and commercial expertise, active partnerships with diverse stakeholders, and a proven record of innovation. These Consortium members will facilitate local yet cross-cutting projects that involve educational institutions, Sea Grant, NOAA assets, industry partners, non-governmental organizations, and local seafood businesses and communities, ensuring that region-specific plans for seafood product development, test bed facilities, and educational programs are consistent across the nation.



BLUE MUSSEL HAEMOCYTE FUNCTION UNDER ENVIRONMENTAL STRESS

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Blue mussels (*Mytilus* spp.) are economically and ecologically significant, they are the most farmed bivalves in Europe representing 35% of total aquaculture production. Climate change is increasing the frequency of extreme rainfall events, causing hyposalinity stress in sea loch aquaculture sites. Mussel immunity depends on haemocyte phagocytosis, but the effects of salinity stress on this critical immune function remain poorly understood. Haemocytes were extracted from Scottish rope-grown *Mytilus* spp. and exposed to salinities of 33, 20 and 13 parts per thousand (ppt) representing standard marine conditions, moderate hyposalinity and severe hyposalinity. Phagocytic activity was quantified using pHrodo™ fluorescent beads conjugated with *Staphylococcus aureus*, *Escherichia coli*, and Zymosan particles over 3-hour live imaging sessions. Additional experiments examined combined salinity and heat shock effects. Cells incubated at marine salinity (33 ppt) sustained stronger long-term phagocytic responses than those at 20 ppt or 13 ppt; severe hyposalinity uniformly suppressed phagocytosis. While mild hyposalinity produced a transient increase in phagocytic activity, likely representing a change between immune function and osmotic stress tolerance. Optimal survival conditions were modelled at 28.4 ppt and 10.4°C. Severe hyposalinity combined with heat stress severely compromised haemocyte function. These findings highlight the vulnerability of mussel aquaculture to climate driven multi-stressor events and suggest that extended observation periods are essential for accurate assessment of phagocytic capacity.

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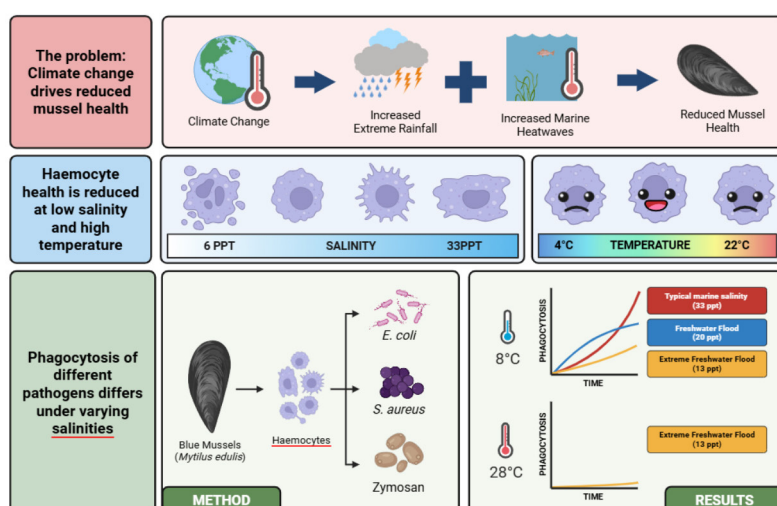


Figure 1: Graphical abstract: Combined salinity and thermal stress from climate change impairs haemocyte health and pathogen phagocytosis in blue mussels (*Mytilus* spp.).

ECOPATH MODELLING IN AQUACULTURE – AN OVERVIEW

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The intensification of aquaculture has largely simplified ecological structures, leading to the dominance of monoculture systems, truncated trophic pathways, and limited contributions of natural food webs to production. However, there is growing interest in more complex systems, such as integrated and multi-species aquaculture, due to their potential benefits in nutrient recycling and resource-use efficiency. Optimizing stocking densities, species assemblages, and feeding strategies in such systems requires a detailed understanding of predator–prey interactions, energy and nutrient flows among functional groups, and overall ecosystem functioning. These challenges highlight the need for analytical frameworks capable of representing multi-species aquaculture systems as ecological networks rather than isolated production units.

Among ecosystem modelling tools, the Ecopath with Ecosim (EwE) framework has become one of the most widely used approaches for analysing aquatic food webs, owing to its balance between simplicity and ecological realism, as well as its relatively low data requirements. Originally developed to construct mass-balanced representations of marine ecosystems^{1,2}, it has been increasingly applied to aquaculture systems. However, aquaculture systems differ substantially from large-scale, open marine ecosystems. They are typically characterized by simplified community composition, short production cycles, and small-scale, engineered environments. Consequently, this emerging field of application raises fundamental methodological questions: To what extent can an Ecopath model, originally designed for natural food webs, adequately represent systems under strong human control? How should models be constructed and parameterized for rapidly changing systems of relatively short duration? Which ecosystem indicators are most relevant for aquaculture management and optimization? To date, no review has systematically examined these challenges or provided an overview of research objectives and system types addressed in aquaculture applications of EwE.

In this review, we aim to provide a comprehensive synthesis of how Ecopath has been applied to aquaculture-related questions across system types and geographic regions. Beyond cataloguing applications, we critically analyse these studies by confronting the conceptual assumptions and modelling capacities of EwE with the structural characteristics of aquaculture systems. We seek to clarify both the opportunities and limitations of EwE in aquaculture science. This work also contributes to the development of ecosystem-based aquaculture management strategies that are both ecologically robust and operationally relevant.

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DEVELOPMENT OF OSMOTIC AND OXYGEN STRESS MODELS TO EVALUATE SHRIMP RESILIENCE IN EXPERIMENTAL SYSTEMS

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Shrimp aquaculture systems frequently experience environmental fluctuations such as salinity shifts and oxygen depletion, which can significantly affect shrimp health, performance, and survival. However, many experimental trials evaluating nutritional strategies or functional feeds are conducted under relatively stable conditions, which may not fully reflect the challenges encountered in commercial farming environments.

This study presents the development of controlled abiotic stress challenge models designed to evaluate shrimp robustness and tolerance under experimental conditions. Two environmentally relevant stressors were investigated: osmotic stress induced by salinity shifts and oxygen depletion through controlled reduction of dissolved oxygen levels. The models were designed to generate reproducible stress responses while maintaining experimental consistency.

Different stress intensities were explored to address various experimental objectives. Acute stress models can be used to rapidly assess shrimp tolerance thresholds, while mild or moderate stress conditions applied over longer periods may allow shrimp to physiologically adapt and reveal more subtle differences among treatments. Such approaches may also be more appropriate for evaluating responses at different shrimp stages or sizes.

The development of standardized abiotic stress challenge models focusing on osmotic and oxygen stress may provide valuable tools for evaluating functional feeds, nutritional interventions, and management strategies aimed at improving shrimp resilience. These models may help bridge the gap between laboratory trials and real farming conditions and support the development of more robust shrimp production systems.

DIETARY PHYTOBIOTIC ADDITIVE UNLOCKS KEY MOLECULAR PATHWAYS DRIVING ACCELERATED WOUND REPAIR IN ZEBRAFISH

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Introduction

Skin integrity is essential for fish health and is the primary biological barrier against environmental stress and pathogens. While the phytobiotic-based feed additive (APEX, Adisseo) has previously been shown to accelerate wound healing and skin regeneration in zebrafish, the underlying mechanisms are not yet fully understood. This study uses transcriptomic analysis to examine how the additive modulates the molecular pathways required for skin repair and barrier restoration.

Material and Methods

Adult zebrafish (*Danio rerio*) were randomly assigned to three groups: uninjured control, wounded control, and wounded with the additive supplementation. The additive was administered orally twice a week for 14 days before wound induction. Precise 2-mm circular skin wounds were created using laser ablation. At Day 10 post-injury, skin tissues were collected to study gene activity during the active repair phase. Transcriptomic profiling was performed using Illumina paired-end RNA-Seq to identify differentially expressed genes (DEGs) based on the criteria of (|Fold Change| > 2, *p.adjust* < 0.005) and to characterize specific biological pathways influenced by the additive during the recovery process.

Results

Supplementation with the phytobiotic-based additive significantly improved wound closure efficiency, which represents an increase of nearly 40% compared to the untreated wounded control group. Transcriptomic analysis showed that untreated fish responded to injury with a broad decrease in homeostatic functions. Specifically, blood coagulation and the angiogenic cluster, including regulators such as *vegfc*, *etv2*, and *robo4*, were suppressed, indicating a disruption of the vascular infrastructure needed for nutrient delivery. In contrast, additive supplementation led to a clear reactivation of these molecular processes. The additive increased the activity of pathways related to the steroid biosynthetic process for cholesterol synthesis and cell membrane repair, blood coagulation, complement activation, and the regulation of wound healing. Furthermore, the activation of the PPAR signaling pathway and the presence of the structural protein HSPG2 show that the tissue moved successfully from the inflammatory phase into organized remodeling.

Conclusion

These findings show that the functional additive accelerates skin healing by restoring blood supply and providing essential metabolites for repair. By activating steroid biosynthesis and signaling pathways for growth, the additive effectively supports recovery, leading to faster wound closure and a return to a healthy state.

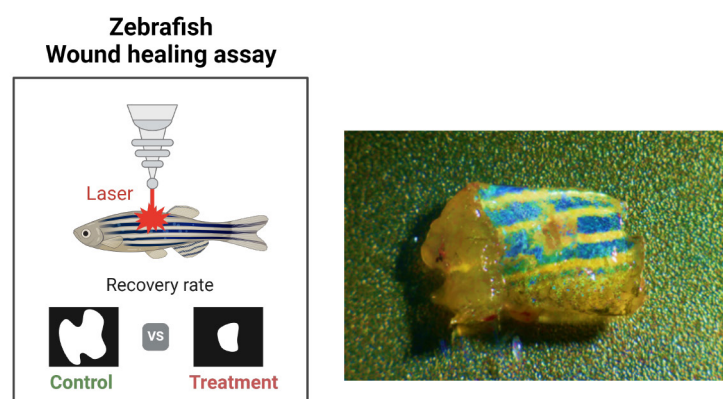


Figure 1. Zebrafish transcriptomic profiling after skin wounding. Precise laser-induced wounds were used to characterize the molecular mechanisms and signaling pathways modulated by the functional additive during skin repair.

MULTITISSUE PROTEOMIC INSIGHTS REVEAL HOW A PHYTOBIOTICBASED ADDITIVE ACTIVATES AN INTEGRATED IMMUNE DEFENSE NETWORK IN GILTHEAD SEABREAM

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Introduction

Fish rely on the complex interaction of innate and adaptive immunity to manage pathogens. However, intensive production conditions create stressors like high stocking densities, temperature fluctuations, and plant-based protein sources that act as catalysts for disease. These factors often lead to chronic inflammation, immune exhaustion, and physiological depression, resulting in significant economic losses. Dietary functional additives offer a preventive strategy to support health and reduce disease severity. This study utilized high-throughput proteomics to underline how a phytobiotic-based additive (Sanacore GM, Adisseo) enhances immunocompetence in fish.

Material and Methods

This trial utilized gilthead seabream (*Sparus aurata*) with an initial body weight of 100g. These fish were reared under optimal conditions at a density of 6kg m⁻³ for 6 weeks until reaching an average size of 160g. To study the proteome profiles, primary immunoregulatory organs were collected: the anterior intestine (frontline barrier), the liver (phagocytic coordination center), and the head kidney (primary immune cell factory). The analysis was performed using shotgun proteomics (LC-MS/MS) and advanced protein network analysis to identify differentially expressed proteins (DEPs) and their associated biological functions.

Results

The proteomic analysis revealed a total of 2,197 annotated DEPs across the three tissues, revealing that the additive provides a holistic reinforcement of the fish's integrated immune defense network. Rather than independent tissue reactions, the phytobiotic-based additive facilitated a synchronized defense system starting at the frontline barrier and extending through systemic regulation. In the intestine, the additive improved early sensing of threats via the antigen presentation pathway and boosted the ability of local cells to neutralize pathogens through phagocytosis before they could enter the body. It also activated the PPAR pathway to maintain gut integrity through antioxidant and anti-inflammatory responses, reducing the overall infection pressure on the fish. The liver supported this effort by organizing the movement of immune cells via cytoskeletal reorganization and utilizing specialized chaperone proteins to keep proteins correctly folded and functional during stress. Additionally, the liver enhanced MHC class I peptide presentation to help the system mount stronger downstream responses. Finally, the head kidney acted as a high-power regulatory center by significantly increasing the production of defense molecules, including antimicrobial peptides (AMPs) and cytokines. It accelerated pathogen recognition through MHC class I and II molecules and triggered targeted apoptosis in infected cells to stop disease from spreading through the body.

Conclusion

Sanacore GM serves as a comprehensive immunoregulator that synchronizes early pathogen detection in the gut with efficient cellular coordination in the liver and powerful systemic execution in the head kidney. By reinforcing physical barriers and accelerating pathogen recognition, the additive ensures a faster and more efficient immune response under production conditions.

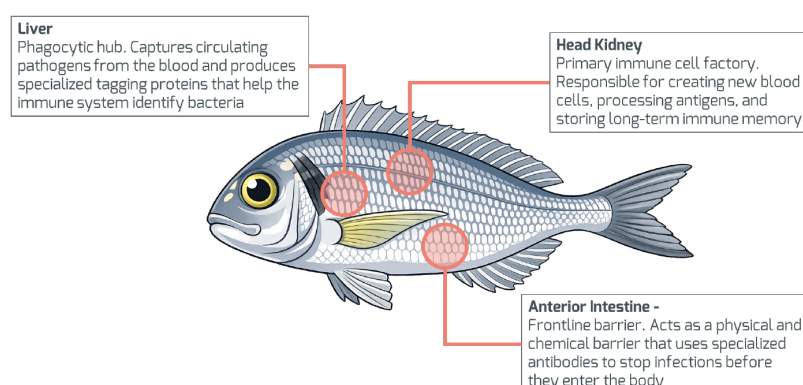


Figure 1. Schematic overview of the three key immunoregulatory tissues analyzed in gilthead seabream.

DEVELOPMENT OF A FIELD-DEPLOYABLE SEQUENTIAL INJECTION ANALYSIS SYSTEM FOR AQUACULTURE MONITORING

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Ammonia nitrogen ($\text{NH}_3/\text{NH}_4^+$) is a critical water quality parameter in aquaculture. Its accumulation can cause physiological stress in aquatic species. This study presents the development of a field-deployable automated monitoring platform based on Sequential Injection Analysis (SIA). The innovation lies in the extreme miniaturization of the fluidic manifold and the integration of a custom-designed signal conditioning unit. By employing an automated air-scouring sequence and a streamlined in-line heating architecture, the platform ensures highly stable reaction conditions while maintaining a compact footprint. Experimental validation demonstrated exceptional analytical sensitivity, achieving a striking 2.52 V peak signal for trace-level ammonia (100 ppb). This synergy between custom electronics and optimized mechanical design provides a highly reliable, low-maintenance tool for real-time aquaculture management.

1. Introduction

Continuous ammonia quantification is essential for precision aquaculture. However, commercial automated sensors often suffer from excessive bulkiness, insufficient trace-level sensitivity, and high maintenance in field environments (Silva et al., 2025). To address these issues, this study develops a field-deployable SIA platform (Mesquita et al., 2020). By miniaturizing core components, the proposed system delivers highly sensitive continuous monitoring with significantly reduced software dependency.

2. Methodology

The system leverages a precision syringe pump (Cavro XCalibur) coupled with a streamlined fluidic manifold to execute fully automated sampling and reagent mixing protocols. As illustrated in the system architecture (**Figure 1A**), the platform utilizes a decoupled thermal control design. A standalone hardware PID controller is implemented to maintain stable reaction temperatures in the holding coil during the 0–420 s incubation phase, independent of the main software operation.

Furthermore, an automated in-line air-scouring step is integrated to continuously purge residual microbubbles from the flow path. For signal processing, the raw analog output from the PMT-based detector (JASCO FP-1520) is directly routed to a custom signal conditioning board. This dedicated circuit performs localized background subtraction and low-pass filtering to ensure signal clarity prior to digitization.

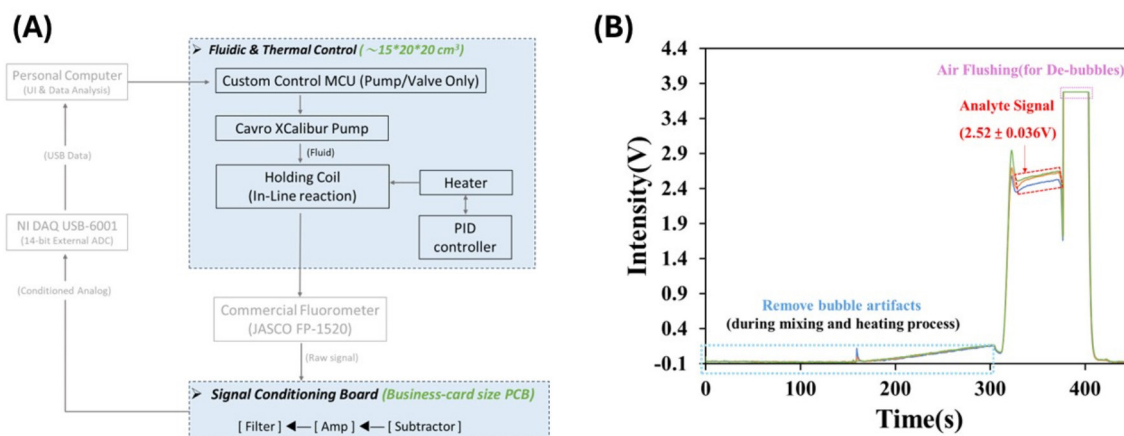


Figure 1. (A) Schematic diagram of the field-deployable SIA platform, detailing the integrated fluidic and electronic modules. (B) Analytical chronogram of five consecutive measurements, showing consistent peak signals and a stable baseline following the air-scouring process.

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3. Results and Discussion

The performance of the system was evaluated through consecutive automated measurements. As shown in the analytical chronogram (**Figure 1B**), the system demonstrated highly stable operation over five cycles ($n=5$). The implementation of the air-scouring mechanism effectively eradicated optical artifacts and baseline drift typically caused by microbubble accumulation. Crucially, the processed analyte signal for a trace ammonia concentration of merely 100 ppb reached a peak intensity of 2.52 V. This high signal-to-noise ratio is primarily attributed to the custom signal conditioning board, which effectively filters environmental interference and stabilizes the baseline without relying on software post-processing. By isolating the fluidic control and thermal acceleration into a streamlined, modular hardware architecture, the platform successfully overcomes the portability and sensitivity bottlenecks of conventional field-deployed sensors.

4. Conclusion

The developed SIA module demonstrates a significant advancement in hardware-centric miniaturization for aquaculture monitoring. The primary contribution of this work is the realization of an ultra-compact monitoring core ($\sim 15 \times 20 \times 20$ cm³) that integrates proprietary signal conditioning electronics for high-fidelity data acquisition at trace levels. By focusing on mechanical streamlining of the heating mechanism and hardware-level noise suppression, the system achieves stable measurements with minimal software intervention. This robust, miniaturized architecture offers a scalable solution for integration into next-generation aquaculture IoT networks.

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AT THE CROSSROADS OF CLIMATE AND DISEASE: AUTOPHAGY COUNTERS NERVOUS NECROSIS VIRUS INFECTION IN *Epinephelus coioides* UNDER HEAT STRESS

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Introduction

The orange-spotted grouper, *Epinephelus coioides*, is one of the most valuable aquaculture species in tropical and subtropical Asia. However, it faces two major challenges, *i.e.* infection by nervous necrosis virus (NNV) and rising heat stress due to climate change (Chen *et al.*, 2010). Both of these stressors disrupt cellular balance and activate two key protein quality control systems, namely the heat shock response (HSR) and autophagy. These systems do not work independently. Instead, they interact closely and influence whether cells adapt and survive, or collapse under stress. In this context, LC3 and HSF1 serve as useful markers. Together, they provide a practical way to study how HSR and autophagy interact during viral infection and thermal stress in fish (Dokladny *et al.*, 2015). Understanding this interaction could lead to new strategies for disease control and aquaculture management, such as targeted antiviral approaches for NNV susceptible species or improved acclimation methods to enhance resilience to warming conditions. Addressing these combined challenges is becoming increasingly important for the aquaculture industry.

Materials and Methods

Orange-spotted grouper larvae, from Taiwanese aquaculture sector, reared in the Translational Center for Marine Biotechnology of National Cheng Kung University were used. Grouper fin epithelium cell line, GF-1, was obtained from the developer and cultured until ~80% confluence before subjected to experimental treatments. The NNV used in the present study was isolated from local diseased fish and prepared according to previous report (Chen *et al.*, 2010). The relative expressions of genes of interest were measured by RT-qPCR, and the protein levels were quantified by immunoblot with specific antibodies. The NNV capsid protein, autophagosome and lysosome signals were determined by immunofluorescence staining and visualized by LSM 780 confocal microscope.

Results and Discussion

To better understand how these protective pathways function under stress, we cloned and characterized the autophagy marker LC3 from *E. coioides* (*osgLC3*). We then examined how it responds to three relevant stress conditions, including low salinity, NNV infection, and high temperature. At the molecular level, *osgLC3* encodes a conserved protein of 126 amino acids, closely related to LC3b proteins found in both Teleostei and mammals. This highlights the strong evolutionary conservation of the autophagy system. Expression analysis showed that *osgLC3* is present across all major tissues, suggesting it plays a general, ongoing role in maintaining protein balance rather than acting only during stress. Under low-salinity conditions (5 ppt), *osgLC3* expression gradually increased, along with a rise in the LC3-II/LC3-I ratio over 72 hours. This confirms that *osgLC3* is a reliable indicator of autophagy activity and shows that hypo-osmotic stress can directly trigger autophagy in groupers. During NNV infection, a different but related pattern emerged. In grouper fin (GF-1) cells, autophagosome formation was observed as early as 6 hours after infection. In infected larvae, *osgLC3* protein accumulated significantly in damaged brain tissue. Both gene expression and protein activation were concentrated in the brain and eyes, which are the main targets of the virus. This spatial pattern indicates that autophagy is activated directly at sites of viral damage. Heat stress added another layer of regulation. Exposure to 36°C enhanced both *HSF1b* and *osgLC3* expression, and overexpressing *HSF1b* alone was enough to stimulate *osgLC3* transcription. This suggests that the HSR can act upstream of autophagy. However, the relationship is not one-way. Increasing *osgLC3* levels suppressed *HSF1b* activity, revealing a negative feedback loop in which autophagy helps regulate its own activation pathway. This balance appears essential to prevent harmful overactivation of either system. The effects of these interactions on viral infection were significant. Enhancing autophagy, either with rapamycin or by overexpressing *osgLC3*, significantly inhibited NNV replication and capsid protein levels. This shows that autophagy acts as an effective antiviral defense in groupers. In contrast, heat stress and increased *HSF1b* expression promoted viral replication. This indicates that HSR and autophagy are not simply parallel responses but can have opposing effects on infection outcomes. Overall, these findings highlight

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autophagy as an important immune defense in fish and reveal a dynamic, two-way regulatory relationship between HSR and autophagy. This interaction helps explain why fish exposed to high temperatures may be more vulnerable to NNV outbreaks. It also suggests practical strategies for aquaculture, such as promoting autophagy to enhance antiviral resistance or selecting for improved thermal tolerance. Together, these approaches could help strengthen grouper farming against both disease and climate related stress.

Acknowledgment

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HYDROXY-SELENOMETHIONINE SUPPORTS SELENIUM STATUS, ANTIOXIDANT CAPACITY, AND RESILIENCE ACROSS FISH SPECIES

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Selenium is an essential micronutrient for mitigating oxidative stress in fish, as it is required for the synthesis of key antioxidant selenoproteins, including glutathione peroxidase (GPx) and thioredoxin reductase (TrxR). Its biological effectiveness depends strongly on the chemical form supplied in the diet. Selenomethionine (SeMet) can be incorporated into body proteins, particularly in muscle, creating a selenium reserve that can be mobilized when antioxidant requirements increase. Analysis of commercial fishmeals showed that SeMet represented only a small and highly variable proportion of total selenium, even in premium-grade fishmeal. This highlights the importance of considering selenium speciation rather than total selenium content alone.

This review summarizes evidence from studies evaluating hydroxy-selenomethionine (OH-SeMet), a pure organic selenium source (Selisseo, Adisseo), in freshwater and marine fish under different dietary and production conditions. Improved selenium status was consistently observed following OH-SeMet supplementation across studies comparing different selenium sources. Muscle selenium deposition increased by up to 80% in gilthead seabream, while increased tissue selenium deposition was also observed in giant grouper. Higher selenium deposition strengthened antioxidant protection by supporting GPx activity and limiting lipid peroxidation. Under chronic heat stress, OH-SeMet increased muscle selenium deposition and reduced lipid peroxidation by 43% in gilthead seabream, demonstrating greater protection under challenging conditions. OH-SeMet supplementation also supported reproductive performance. In rainbow trout broodstock, higher tissue selenium increased selenium transfer to oocytes and swim-up fry, supported GPx expression and activity in progeny, and improved spawning, hatching success, and early survival. Stronger antioxidant protection also helped preserve final product quality, particularly under stressful conditions. In gilthead seabream exposed to chronic heat stress, OH-SeMet improved fillet whiteness, maintained freshness during ice storage, and enhanced cooked fillet texture.

Together, these findings demonstrate that the value of dietary selenium depends not only on its total concentration but also on its source and bioavailability. By building a mobilizable tissue reserve, OH-SeMet strengthens the antioxidant system and supports reproductive performance, product quality, and resilience under challenging farming conditions

IMPROVING HEAT-STRESS RESILIENCE IN EUROPEAN SEABASS WITH A HEALTH-PROMOTING ADDITIVE

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Rising seawater temperatures are becoming an important challenge for aquaculture. Marine heatwaves present an even greater risk, as sudden temperature increases raise metabolic demand while reducing appetite. Fish therefore prioritize energy for stress response and survival, leaving less energy for growth, immunity and recovery. High temperatures can also weaken intestinal integrity, reducing nutrient utilization and increasing health risks.

This study evaluated the efficacy of a functional phytobiotic-based blend of antioxidant ingredients formulated to support fish under high-temperature stress (HeatPro, Adisseo). European seabass (IBW 144 g) were distributed across six tanks in a RAS system, with 14 fish per tank, and assigned to a control or additive-supplemented diet. Fish were fed to satiation twice daily for 12 weeks. They were acclimated at 26°C for 1 week, followed by 8 weeks at 28°C. Heat stress was then induced at 32°C for 1 week, followed by 7 days at 28°C and 7 days at 30°C to simulate temperature fluctuations under farming conditions. Assessments included survival, growth performance, feed conversion ratio, plasma cortisol, glucose and lactate, and hepatic MDA, HSP70 and IGF-1 expression and esterase activity.

Under high-temperature stress, the additive increased survival by 8% compared with the control. Supplemented fish had lower plasma cortisol, glucose and lactate, together with lower hepatic MDA and HSP70 expression, indicating reduced physiological, metabolic, oxidative and cellular stress. Higher IGF-1 expression and esterase activity were also observed. These responses were accompanied by a 12% increase in individual weight gain, a 17% improvement in specific growth rate and a 20% reduction in feed conversion ratio.

Overall, supplementation supported antioxidant defense, reduced the energy required to cope with heat stress and preserved more energy for growth, resulting in better survival, growth performance and feed efficiency.

TEMPERATURE TRENDS IN FISH PONDS IN LORRAINE (FRANCE) FROM 1998 TO 2025

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Introduction

Over the past four decades, Europe has warmed at twice the global average pace, becoming the continent with the fastest heating (Copernicus Climate Change Service (C3S), 2025). If the link between atmospheric warming and water body warming is well established on a global scale, a range of other local factors (area, depth, turbidity, watershed morphology, shading, and exchanges with the water table) also play a role in shaping water temperature dynamics (O'Reilly et al., 2015), the variability of which remains poorly characterized for fish ponds.

With 23% of surface area in 2018, the Grand Est region (North-East of France) was the second-largest French region in pond surface area, mainly due to the Moselle department (DRAAF Grand Est, 2021). The latter, with a semi-continental climate, is not escaping current global warming. Therefore, increasing temperature imposes limits on freshwater biodiversity (Lamouille-Hébert et al., 2024). In poikilothermic fish, temperature directly influences key biological functions, including metabolism, ingestion, digestion rate, and reproduction cycles (Souchon and Tissot, 2012). Rising water temperature can also indirectly affect fish population by reducing dissolved oxygen levels, increasing ammonia concentrations, and degrading water quality (Mugwanya et al., 2022; Reid et al., 2019). Together, these effects may favour certain species over others, with potential repercussions for fish farming activities.

In this context, the objective of this study was to analyse the evolution of the thermal regimes of eight fish ponds belonging to the “Domaine de Lindre” between two periods of eight years each separated by 20 years (1998–2005 and 2018–2025) (Hirtz & Gentilhomme).

Materials and methods

Eight ponds within the Seille watershed (4.8 to 599.4 ha, mean depth 1–2 m), hydraulically connected, were selected for their size gradient and the availability of regular thermal records (measures every two or three weeks). Water temperatures (probes mounted on dikes, 60–70 cm depth) were compared between two periods 20 years apart (1998–2005 and 2018–2025), along with daily meteorological data from the nearest Nancy-Essey weather station. Annual thermal cycles were modelled using sinusoidal fitting for each site and year, allowing the extraction of biologically relevant indicators: annual extremes, thermal amplitude, and duration below/above critical thermal thresholds related to cold chilling winter period and hot summer period (thermal stress). Inter-period changes were tested using Spearman's correlation and Wilcoxon tests. Mixed linear models were used to identify which the meteorological factors and/or pond surface area best explained water warming.

Result

The thermal trajectories varied significantly among the ponds. Lindre, the largest pond, showed the most pronounced warming (+1.89 °C mean annual temperature, +1.39 °C seasonal amplitude), while the smallest pond (Armeschouss) cooled. Warming and amplitude changes were strongly correlated with pond surface ($q = 0.929$ and 0.905 , respectively, both $p < 0.01$). Lindre alone showed significant reductions in cold-threshold duration (<9 – 12 °C) and significant increases of the periods above 15 °C and 22 °C, indicating shorter cold periods and longer thermophilic development windows. The best mixed model ($R^2 = 94.3\%$) identified the 3-day mean air temperature as the dominant predictor of water temperature, followed by sunshine duration, with minor site-specific effects.

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Discussion

The strong area-dependence of long-term warming ($q > 0.9$) suggests that pond size acts as a genuine climatic integrator rather than a confounding factor: thermal inertia dampens short-term noise in large ponds, revealing decadal trends more clearly, whereas small ponds remain dominated by local hydrology. This scale-dependence also explains why area had no significant effect in the daily mixed model ($R^2 = 0.943$, air temperature-driven), atmospheric forcing governs short-term variability, whereas pond morphology shapes how this forcing translates into long-term trends. Biologically, the asymmetric seasonal warming creates a trade-off: shortened cold spells jeopardize winter vernalization, whereas longer warm periods benefit thermophilic species but raise thermal-stress and eutrophication risks. Under the TRACC scenarios, a projected warming of +2.1 °C by 2050 and +3.3 °C by 2100 (Météo-France, 2026) is expected to intensify these opposing pressures unevenly across the pond network. These findings provide a quantitative basis for a broader project experimentally assessing how such thermal shifts affect gametogenesis, stress, and reproductive success in several fish species of aquaculture interest.

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FIRST GENERATIONS OF DOMESTICATION: A MULTIGENERATIONAL MONITORING OF PHENOTYPIC CHANGES IN ZEBRAFISH *Danio rerio*

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Domestication is an evolutionary process in which the target species must adapt to new captive environmental conditions, resulting in phenotypic and genetic changes (Milla et al., 2021). Some of these changes are recurrent across various mammalian and avian species, constituting the domestication syndrome (Wilkins, 2020). Although similar changes exist (Milla et al., 2021), this syndrome rarely includes teleost fish. Yet, domestication is a major bottleneck for the diversification of aquaculture and the mechanisms underlying the early adaptation of fish to captive environments remain poorly understood, as studies often focus on a single biological function and do not examine the kinetics of these changes. Unlike terrestrial livestock, wild teleost populations are still accessible, providing a unique opportunity to investigate the first stages of domestication and the emergence of domestication-associated phenotypes. Using zebrafish (*Danio rerio*) as a model species, we investigated how key phenotypic traits change during the first generations of captivity and whether these changes converge with those observed in long-domesticated laboratory strains.

Our study combined two complementary approaches: (i) a comparison of four laboratory strains (AB, SJD, TU and WIK) representing independent domestication histories, and (ii) a multigenerational monitoring of a wild-derived population from the founder generation (F0) to the third captive generation (F3). We evaluated a broad range of traits associated with domestication and aquaculture performance, including growth, morphology, behaviour, stress physiology and reproduction.

These approaches highlighted early changes but also revealed that certain traits were expressed differentially later in the process. For instance, the early generations expressed stress-related behaviours, while the domesticated reference population was more aggressive and bolder. These differences persisted at a later stage, with variations in aggression levels among domesticated strains. Likewise, reproductive performance differed between F0 and its descendants (better fertilisation rates and sperm quality) and among domesticated strains. This also applied to morpho-anatomical traits (body weight, relative brain weight). In contrast, other traits showed variations in the early generations but not between the strains, suggesting that the adaptation process may have stabilised. This was the case for pigmentation (decrease between F0 and F2) and basal cortisol levels (increase between F0 and F3). Except for serotonin levels and puberty in males, all measured traits showed significant differences between the early generations or with the domesticated population.

Overall, our results demonstrate that domestication induces rapid but trait-specific phenotypic changes, with distinct temporal trajectories across biological functions. Some traits appear to reflect a common adaptive response to captivity, whereas others remain strongly influenced by domestication history, founder effects or selective breeding. These findings provide one of the first comprehensive descriptions of the kinetics of early fish domestication and offer valuable insights for improving the domestication of emerging aquaculture species.

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LIVE FOOD MICROBIAL MANAGEMENT AND ITS ROLE IN CONDITIONING THE LARVAL REARING ENVIRONMENT

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Introduction

Microbial management in hatcheries is particularly critical during the early larval stages, when larvae are highly sensitive to microbial imbalances and the tank microbiome is still developing. This instability increases the risk of microbial disruptions and opportunistic bacterial proliferation. Beyond pathogen suppression, modern microbial management strategies therefore focus on steering the early establishment of beneficial microbial communities that support water quality, enhance microbial stability and promote larval health. This study evaluated disinfection and MICF-based microbial management strategies during live food production (rotifers and *Artemia*) and assessed their impact on the larval rearing environment and seabream larval performance.

Materials and methods

Identification of Sanolife MIC-F *Bacillus* strains: Pure cultures of the *Bacillus* strains (*B. subtilis*, *B. licheniformis*, and *B. pumilus*) were plated on Tryptic Soy Agar (TSA) and incubated at 37 °C for 24 h. Prior to the start of the trial, strain identity was confirmed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

Rotifer treatments: L-type rotifers were cultured on a diet consisting of 85% fresh baker's yeast and 15% Roboost and Vitalgae Nanno. Prior to larval feeding, rotifers were enriched with Easy Dry Selco (EDS). Following enrichment, rotifers were subjected to one of two treatments:

1. **Control treatment:** no use of Sanolife MICF and Sanocare SURE.
2. **Selective microbial management:** a 20min bath applying Sanolife MICF at 2.5 g L⁻¹ together with Sanocare SURE at 1 g L⁻¹ to steer the microbiota of the rotifers

The microbial community associated with rotifers was analyzed using culture-based and molecular methods. Bacterial abundance was determined by plate counts on TSA and on TCBS (Thiosulfate-Citrate-Bile Salts-Sucrose) agar. The amount of *Bacillus* spp. present in the sample were estimated by plating the sample on TSA at 37 °C for 24 h. Additionally, Illumina 16S rRNA gene sequencing was used to identify dominant bacterial phyla.

Artemia treatments: Three production protocols were evaluated. In all treatments, nauplii were produced using EG SEP-Art® or EG SEP-Art® D-FENSE, followed by specific post-hatch management:

1. **Control:** Artemia produced using EG SEP-Art® without additional microbial management
2. **SEP-Art D-FENSE-based management:** EG SEP-Art® D-FENSE cysts were hatched and transferred after 21 h to enrichment tanks, where enrichment was performed using EDS and a water conditioner.
3. **SEP-Art D-FENSE followed by microbial enhancement:** Artemia were produced as in treatment 2. After enrichment, nauplii were rinsed, concentrated to 5 million L⁻¹, and incubated for 30 min with germinated *Bacillus* (Sanolife MIC-F activated for 1 h at 30 °C in sterile 25 ppt water), followed by cold storage with 250 ppm peracetic/peroxide-based sanitizer.

As for the rotifers, identical analyses were conducted using culture-based bacterial plating and Illumina-based 16S rRNA gene sequencing to characterize associated microbial communities.

Seabream larval rearing: Larval rearing was conducted under three live-food-derived microbial management strategies:

1. **Negative control:** live food produced without microbial management.
2. **Positive control:** live food produced using microbially managed rotifers and EG SEP-Art® D-FENSE-based Artemia production.
3. **Microbial control:** live food produced under microbial management protocols combined with MIC-F microbial enhancement.

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For each treatment, two replicate 6,000L tanks were stocked with 600,000 justhatched gilthead seabream (*Sparus aurata*) larvae. Greenwater conditions were maintained during the first 30 dph by daily addition of VitAlgae Nanno (60–70 g.m⁻³). Larvae were fed treated rotifers from 3 to 30 dph, AF from 16 to 26 dph, and enriched EG SEP-Art® (D-FENSE) Artemia from 22 to 53 dph. A commercial dry diet (O.Range, INVE Aquaculture, Thailand) was introduced at 19 dph and fed until weaning at 55 dph. After weaning, medium-sized larvae were selected and monitored for an additional three weeks of post-weaning.

Tank biofilm bacterial abundance was evaluated at 30 and 56 dph by swabbing 5 cm² of tank surface, followed by homogenization and plating.

Larval performance was assessed based on survival, growth, stress resistance, deformity incidence, and fatty acid methyl ester (FAME) profiles. At 55 dph, larvae were graded to determine fry survival and quality across treatments.

Results

No significant differences in larval growth or quality were observed among treatments, but clear differences emerged in the microbial profiles of Artemia and tank biofilms. Disinfection-based treatments produced significantly cleaner Artemia, with ~2log reductions per nauplius in both total bacteria and *Vibrio* load. Probiotic *Bacillus* strains were effectively incorporated, averaging $\sim 1.6 \times 10^3$ bacilli per nauplius after 4 h of cold storage in treated Artemia. In tank biofilms, *Bacillus* spp. persisted up to 30 dph when MICF was applied to rotifers, and up to 56 dph at higher levels ($\sim 10^5$ bacilli per 0.5 cm²) when MICF was supplied via both rotifers and Artemia.

Discussion

These findings support the view that microbial management in larval hatcheries contribute to environmental conditioning. By facilitating the early establishment and maintenance of beneficial microbial communities, *Bacillus*-based microbial management strategies contribute to enhanced system stability and resilience. Such advantages are likely to become most evident under suboptimal or stressful rearing conditions, where microbial balance plays a critical role in larval health.

INTEGRATED MONITORING, METABOLOMIC PROFILING, AND MICROBIAL COMMUNITY ANALYSIS OF LAND-BASED CULTIVATION OF *Ulva reticulata* FOR BIO-BASED MATERIAL PRODUCTION

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Introduction

Green macroalgae of the genus *Ulva* have considerable potential as sustainable sources of food, feed, and functional biomaterials. However, reliable land-based cultivation requires optimized environmental conditions and a comprehensive understanding of the physiological, metabolic, and microbial changes occurring during growth. This study aimed to determine suitable cultivation conditions and establish an integrated monitoring framework for the land-based cultivation of *Ulva reticulata*. Its potential as a source of high-value functional compounds was also evaluated.

Materials and Methods

Wild *Ulva* specimens collected from the coastal waters of Jeju Island, Republic of Korea, were isolated and maintained under controlled land-based cultivation conditions. Species identity was confirmed as *U. reticulata* using internal transcribed spacer and **rbcl** sequence analyses. Growth responses were evaluated at nutrient concentrations corresponding to 0–200% Provasoli's enriched seawater medium and irradiance levels ranging from 0 to 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Biomass production, specific growth rate, and the maximum quantum yield of photosystem II, expressed as Fv/Fm, were measured to determine suitable cultivation conditions. Water temperature, salinity, pH, turbidity, cultivation images, biomass, Fv/Fm, and nutrient concentrations were collected and integrated into a time-series dataset. Untargeted metabolomic profiling was conducted using liquid chromatography–tandem mass spectrometry with C18 and amide columns. Bacterial communities in the cultivation water of blade- and cluster-form cultures were analyzed using 16S ribosomal ribonucleic acid gene sequencing.

Results

The highest growth performance and stable photosynthetic activity were observed at 100% nutrient concentration. Irradiance levels of 50–100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ supported efficient growth, with the highest productivity observed at 100 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Image-based analysis indicated that green color intensity and mean brightness were more promising proxies for biomass variation than projected area alone. Untargeted metabolomic analysis detected approximately 17,000–18,000 metabolic features and more than 3,000 putatively annotated metabolites. Amino acid- and peptide-related compounds, lipids, and organic acids were predominant. Phenylalanine, threonic acid, gulose, 2'-deoxyguanosine, and oxidized fatty-acid derivatives were identified as potentially valuable compounds for industrial applications. Blade- and cluster-form cultures shared approximately 70% of high-confidence metabolites. However, cluster-form cultures exhibited greater metabolite diversity and higher relative levels of several amino acid-, peptide-, nucleotide-, and lipid-related metabolites. Distinct bacterial community structures were also observed in the cultivation water. Rhodobacterales–*Marivita*-related taxa predominated in blade-form cultures, whereas Flavobacteriia–*Winogradskyella*-related taxa were relatively abundant in cluster-form cultures.

Discussion

The results indicate that nutrient and irradiance conditions are important factors affecting the productivity and physiological stability of *U. reticulata* under land-based cultivation. Image-derived color parameters may provide a non-destructive approach for monitoring biomass changes, although further calibration using directly measured biomass is required. The differences in metabolite profiles and bacterial communities between blade- and cluster-form cultures suggest that morphological development is associated with changes in both algal metabolism and the cultivation-water microbiome. The higher abundance of amino acid-, peptide-, nucleotide-, and lipid-related metabolites in cluster-form cultures may indicate increased metabolic activity and microbial transformation of organic matter. However, further controlled experiments are required to determine causal relationships between microbial community structure, algal morphology, and metabolite production. Overall, the integration of environmental, imaging, physiological, metabolomic, and microbial data provides a foundation for developing predictive models for land-based cultivation and for controlling the production of functional compounds from *U. reticulata*.

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COMPARATIVE PHARMACOKINETICS AND WITHDRAWAL TIME OF FLUMEQUINE IN NILE TILAPIA (*Oreochromis niloticus*) AND ASIAN SEABASS (*Lates calcarifer*) FOLLOWING ORAL FEED ADMINISTRATION

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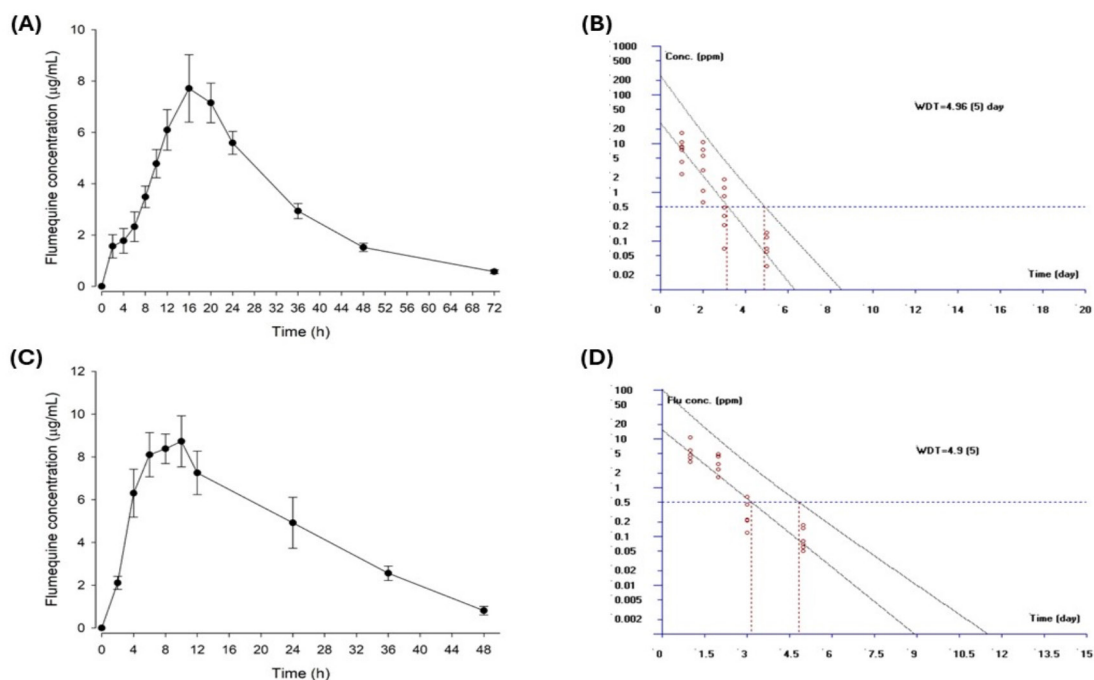
Introduction

Quinolones are often used in the livestock and fish farming industries to treat bacterial infections. Flumequine (FMQ), the first fluoroquinolone with antimicrobial activity against a wide range of Gram-negative bacteria, was approved for use in food fish in the EU, China, Vietnam, and Taiwan. The pharmacokinetics (PK) of FMQ have been studied in several fish species, including Atlantic salmon (*Salmo salar*), cod (*Gadus morhua*), goldsinny wrasse (*Ctenolabrus rupestris*), halibut (*Hippoglossus hippoglossus*), turbot (*Scophthalmus maximus*), eel (*Anguilla anguilla*) (Hansen et al., 2000), blunt snout bream (Xu et al., 2016), and lumpfish (Haugland et al., 2019). However, no data are available on its PK and withdrawal time (WDT) in Nile tilapia and Asian seabass, two of the most important farmed fish species in Taiwan. The objectives of the current study were to determine the PK characteristics of FMQ in both species reared at 25°C following a single oral dose of FMQ via medicated feed (20 mg kg⁻¹ b.w.). Tissue depletion and the appropriate WDT of FMQ were also studied following daily oral dosing for five days.

Materials and methods

Twenty-four healthy Nile tilapia (250 ± 50 g) and 24 Asian seabass (200 ± 50 g) were obtained from a commercial aquaculture facility. The fish were reared in freshwater at 25 °C in an indoor system. For the PK study, 4 fish were orally administered a single 20 mg kg⁻¹ dose of FMQ via medicated feed. Blood samples were collected at 2, 4, 6, 8, 10, 12, 16, 20, 24, 36, 48, and 72 h post-treatment for Nile tilapia, and at 2, 4, 6, 8, 10, 12, 24, 36, and 48 h post-administration for Asian seabass. For the tissue-residue study, 20 fish were fed medicated feed at a dose of 20 mg kg⁻¹/day for 5 days. Skin-on-muscle tissues (muscle/skin) were collected from 5 fish per time point on days 1, 2, 3, and 5 post-treatment. FMQ levels in serum and muscle/skin were analyzed by high-performance liquid chromatography with fluorescence detection (HPLC-FLD) as previously described (Rodríguez et al., 2021). PK data were modeled using a one-compartmental framework and analyzed with PKSolver 2.0 under a 1/C weighting scheme. The WDT was calculated from FMQ concentrations in muscle/skin using WT 1.4 software, following EMA guidance (2018).

Figure 1. Serum FMQ concentration-time profile (mean ± SEM) and plot of WDT on Nile tilapia (A and B) and Asian seabass (C and D) following medicated feed at 20 mg/kg and reared at 25°C.



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Results

The serum concentration-time profiles and the plots of WDT calculation are presented in Fig. 1. Following a single oral dose of 20 mg/kg FMQ via medicated feed, the elimination half-life ($t_{1/2k}$), maximum serum concentration (C_{max}), time to reach C_{max} (T_{max}), and the area under the serum concentration-time curve (AUC) in Nile tilapia were calculated to be 12.15 ± 1.07 h, 4.72 ± 0.46 $\mu\text{g/mL}$, 17.03 ± 1.49 h, and 214.19 ± 11.58 h $\cdot\mu\text{g/mL}$, respectively. In Asian seabass, the $t_{1/2k}$, C_{max} , T_{max} , and AUC of FMQ were 11.2 ± 1.22 h, 8.55 ± 0.86 g/mL, 9.25 ± 2.15 h, and 261.02 ± 48.62 h $\cdot\mu\text{g/mL}$, respectively. After five consecutive daily doses of FMQ, the minimum serum concentration at steady state ($C_{min(ss)}$) in Asian seabass was 4.77 ± 3.54 $\mu\text{g/mL}$, significantly lower than in tilapia (10.84 ± 5.86 $\mu\text{g/mL}$). In the drug depletion study, FMQ concentrations in the muscle/skin of Nile tilapia and Asian seabass on Day 1 of drug withdrawal were 8.33 ± 4.56 and 5.73 ± 2.99 $\mu\text{g/mL}$, respectively, and quickly fell below the MRL of 0.5 $\mu\text{g g}^{-1}$; the WDT in both fish species was determined to be 5 days.

Discussion

After administration with medicated feed, the T_{max} for FMQ in Asian seabass was 9.25 h, indicating faster absorption than in Nile tilapia (17.03 h) but slower than in Atlantic salmon (Martinsen et al., 1995) and lumpfish (Haugland et al., 2019), where T_{max} values ranged from 6 to 8 h under similar condition. At a dosage of 20 mg kg^{-1} , the C_{max} of 8.55 $\mu\text{g/mL}$ for FMQ in Asian seabass was significantly higher than in Nile tilapia (4.72 $\mu\text{g/mL}$) and gilthead sea bream (1.7 $\mu\text{g/mL}$; Rigos et al., 2003). In contrast, the $C_{min(ss)}$ of FMQ in Asian seabass was significantly lower than in tilapia. Similarly, at 24 h of drug withdrawal, the level of FMQ residue in the muscle/skin of tilapia was higher than that of Asian seabass. By Day 3, the FMQ concentration in Asian seabass had decreased to below the maximum residue limit ($\text{MRL} < 0.5$ $\mu\text{g g}^{-1}$), while the FMQ concentration in tilapia fell below the MRL on Day 5. This study is the first to comprehensively investigate the PK and WDT of FMQ in Nile tilapia and Asian seabass. The findings indicate clear species-specific PK differences, with faster clearance in Asian seabass and greater persistence in tilapia. Accordingly, dosing and withdrawal times should be species-tailored to ensure efficacy and regulatory compliance.

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COMBINATIONS OF FLORFENICOL AND THIAMPHENICOL ARE SYNERGISTIC AGAINST *Streptococcus iniae* AND *Streptococcus agalactiae* IN NILE TILAPIA

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Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the most important aquaculture species worldwide. However, intensifying aquaculture with high stocking densities and high feeding rates has increased tilapia's vulnerability to bacterial infections, particularly streptococcosis. Florfenicol (FF) and thiamphenicol (TAP), both amphenicol antibiotics, are broad-spectrum antibiotics commonly used in veterinary or aquacultural practice. Previous studies have shown that a synergistic combination of FF and TAP is effective against several bacterial pathogens of swine, avian, and bovine origin (Rattanapanadda et al., 2019; Wei et al., 2016), but this combination has seldom been documented in aquaculture. Thus, the aim of this study was to evaluate potential synergistic effects of FF in combination with TAP against clinical isolates of *Streptococcus iniae* and *Streptococcus agalactiae* from sick tilapia, and to assess whether such combinations might serve as a novel *in vivo* therapy.

Materials and methods

Twenty clinical isolates of pathogenic bacteria from the Laboratory of Clinical Microbiology of Aquatic Animals at the National Pingtung University of Science and Technology were used to determine *in vitro* susceptibility to TAP and FF. The minimal inhibitory concentrations (MICs) were determined using the broth microdilution method as described in the Clinical and Laboratory Standards Institute guidelines (CLSI 2020). Checkerboard microdilution assay and Time-kill experiments were used to determine the synergistic effects of FF+TAP combinations, in accordance with guidelines from the National Committee for Clinical Laboratory Standards (NCCLS 1999). To determine the minimum serum concentrations of FF and TAP at steady state ($C_{\min(ss)}$), 21 healthy Nile tilapia (250±50 g) were allocated to three groups and orally administered FF solution (10 mg kg⁻¹), TAP solution (20 mg kg⁻¹), or FF+TAP solution (5+10 mg kg⁻¹) daily for 5 consecutive days, respectively. On days 1, 2, 3, 4, 5, 7, 9, and 12 after the first administration, a blood sample was collected from each fish. Concentrations of FF in the serum were determined using the HPLC-FLD method described by Wu et al. (2026).

Results

Antibiotic susceptibility was tested by broth microdilution, and the results indicated that 38% of *S. iniae* and 67% of *S. agalactiae* isolates were resistant to TAP (MIC ≥ 8 g/mL); however, all *S. iniae* and 58% of *S. agalactiae* isolates were sensitive to FF (MIC ≤ 4 µg/mL). The checkerboard microdilution assays and time-kill experiments showed a synergistic effect (a fractional inhibitory concentration index (FICI) ≤ 0.5) in 50% of *S. iniae* and 50% of *S. agalactiae* isolates. In addition, the $C_{\min(ss)}$ of FF and TAP in Nile tilapia orally administered a reduced dose of FF+TAP combination were 1.36 ± 0.24 and 2.2 ± 0.2 µg mL⁻¹, respectively. In comparison with those in fish treated with FF at 10 mg kg⁻¹ (2.63 ± 0.33 µg mL⁻¹) and TAP at 20 mg kg⁻¹ (3.7 ± 0.64 µg mL⁻¹).

Discussion

Combination therapy is occasionally used to broaden the antibacterial spectrum and slow the emergence of resistant bacteria, while allowing dose reduction to improve fish safety and reduce environmental impact. In the present study, the FF+TAP combination demonstrated *in vitro* synergy (FICI ≤ 0.5) in 50% of *S. iniae* and *S. agalactiae* isolates. Although the $C_{\min(ss)}$ of each drug under combination therapy was lower than that observed with their respective single-agent regimens, the concentrations of FF (1.36 ± 0.24 µg mL⁻¹) and TAP (2.2 ± 0.2 µg mL⁻¹) may still contribute to therapeutic efficacy with a synergistic mechanism. Notably, the combined exposure of FF+TAP remained higher than that achieved by either agent alone, further supporting the potential for maintained antibacterial activity. FF exposure decreased proportionally at half dose, whereas TAP showed a smaller reduction, suggesting a PK interaction, possibly via FF-mediated CYP3A inhibition. Nevertheless, the overall dose reduction is expected to facilitate faster residue depletion and thus shorten WDT. Accordingly, the FF+TAP combination represents a promising dose-sparing strategy; however, its therapeutic performance under reduced dosage should be confirmed by *in vivo* challenge studies.

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Acknowledgment

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EFFECTS OF BLACK SOLDIER FLY (*hermetia illucens*) MEAL ON LARGEMOUTH BASS (*micropterus salmoides*) GROWTH AND HEALTH IN RECIRCULATING SYSTEMS

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Methodology

The aquaculture industry is increasingly seeking sustainable alternatives to traditional protein sources. This study evaluated the effects of an experimental diet containing insect meal on the growth performance, feed efficiency, yield indices, and tissue morphology of juvenile Largemouth Bass (*Micropterus salmoides*) in a recirculating aquaculture system. Two experimental diets were formulated with identical compositions, except that one included 15% Black Soldier Fly (*Hermetia illucens*) meal as a replacement for soybean meal used in the scientific control. Both experimental formulations were compared against a standard commercial control. A total of 400 fish (70.7 ± 17.9 g) of mixed sex were randomly distributed into nine tanks (50 fish per tank), with three replicate tanks per dietary treatment. Fish were hand-fed once daily at 1.5% of body weight, five days per week for five months under natural photoperiod at a mean water temperature of 21.5 °C. Furthermore, a carbon footprint assessment was conducted using literature and database data.

Results and Discussion

Both experimental diets significantly outperformed the commercial control in terms of final weight and length, while no significant differences were observed between the BSF-inclusive diet and the experimental control. This indicates that BSF meal performs as effectively as a standard high-quality formulation. Feed efficiency was notably improved in both experimental groups, characterized by a lower feed conversion ratio (FCR) and a higher specific growth rate (SGR). While somatic indices showed enhanced fillet yield in experimental groups, histological analysis confirmed a satisfactory health status across all treatments. Intestinal histomorphometry remained unaffected by diet, proving that BSF meal maintains intestinal integrity. Furthermore, reduced hepatocyte vacuolization in both experimental groups suggests improved hepatic cellular integrity compared to the commercial control. The environmental assessment demonstrated that BSF meal inclusion reduced the carbon footprint by approximately 20% (2.09 vs 2.66 kg CO₂ eq. kg⁻¹ feed). These results highlight BSF meal as a sustainable, high-performance protein source that matches the efficacy of standard experimental diets while offering significant environmental benefits for *M. salmoides* production.

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REAL-TIME CAMERA-BASED ANALYSIS OF SPAWNING BEHAVIOUR IN *Sander lucioperca* FOR ARTIFICIAL REPRODUCTION

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Introduction

Computer vision and deep learning methods, particularly convolutional neural networks (CNNs), are increasingly used in precision aquaculture for analysing fish behaviour and appearance. These approaches have been successfully applied to tasks such as feeding monitoring, welfare assessment, and behavioural classification in tanks and sea cages (Føre et al., 2018; Yang et al., 2020). However, the vast majority of existing studies rely on offline processing, in which video data are first recorded and then analysed. Even though many works report that their methods are capable of real-time performance, this is typically demonstrated on pre-recorded datasets rather than during ongoing experiments (Zhao et al., 2019). As a result, these approaches do not allow immediate access to behavioural parameters or intervention during the experiment, and they require the recording and storage of large datasets. In contrast, online/real-time (where analysis is performed during the experiment performance) data processing has been experimentally shown in other domains to enable closed-loop systems, adaptive control, and fundamentally new experimental paradigms (Stowers et al., 2017). Online processing can be performed on-site using specialised low-cost GPU units, without the need to store or transmit data. Such capabilities allow researchers to monitor ongoing processes, detect unexpected events, and dynamically adjust experimental conditions. Despite these advantages, online processing remains largely underutilised in aquaculture research.

In this study, we demonstrate the feasibility and potential benefits of online video analysis in aquaculture by detecting and precisely localising spawning events for artificial pikeperch reproduction.

Materials and Methods

Spawning experiments were recorded using 9 IP cameras under controlled conditions. The fish pair was kept in 1m³ experimental tank with low-light underwater illumination and an artificial nest for several days. The fish exhibits a rotational pattern over the nest during the pre-spawning and spawning periods. Video streams from all tanks were recorded and observed by trained personnel to detect the egg release event. The spawning event is characterised by a short-term increased speed of fish-pair rotation and a curved fish body shape. The video streams were processed using an embedded low-cost computing platform, NVIDIA Jetson Nano. Fish detection was performed using the YOLOv8n model to predict oriented bounding boxes, enabling the estimation of fish position and orientation even under challenging conditions (e.g., overlap and low illumination). The model was trained from existing experiments and applied to the new experiment in the online/real-time regime.

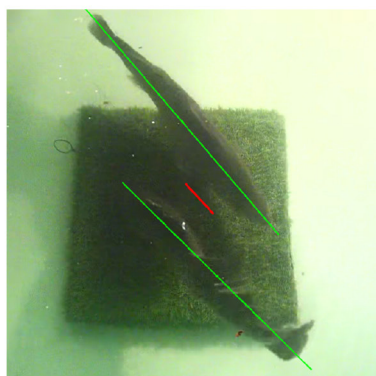


Figure 1. Example of rotational activity processing. Green lines represent fish orientation. The red line indicates that the fish are close to each other, parallel, and it is used for pair rotation velocity calculation.

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Spawning behaviour was determined by analysing the mutual rotation of fish pairs above the nest. The angular change between consecutive frames was computed and transformed into rotational velocity. To robustly capture behavioural dynamics, the signal was smoothed using two temporal windows (3 s and 20 s). Spawning events were detected when the difference between short-term and long-term rotational activity exceeded a defined threshold, reflecting a rapid increase in spinning behaviour preceding spawning (see Fig 1).

The method was evaluated on annotated video recordings containing both confirmed spawning events and control sequences without spawning and during the spawning experiment.

Results and Discussion

The proposed approach successfully detected spawning events across multiple recordings and streaming, while no false detections were observed in control sequences without spawning. A short delay (~3 s) was introduced due to temporal smoothing, which remains acceptable for real-time applications. Immediately after the spawning detection, the eggs were extracted from the fish and used for artificial reproduction. This approach increases the accuracy of the pikeperch reproduction.

This work illustrates the broader advantages of transitioning from offline to online analysis. Online/real-time processing provides immediate access to behavioural parameters, enabling continuous monitoring and allowing researchers to intervene during the experiment. This can improve experimental efficiency, reduce data redundancy, and support adaptive experimental design. The presented fish spawning detection serves as a proof of concept demonstrating that current computational resources already allow online analysis of complex behavioural events in aquaculture. The transition from offline to online processing has the potential to significantly enhance both experimental research and practical applications in precision aquaculture.

Acknowledgment

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FINDING THE BALANCE: EFFECT OF FEEDING LEVEL ON GROWTH, CONDITION AND BODY COMPOSITION OF SUBADULT BURBOT *Lota lota* IN RECIRCULATING SYSTEMS

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Introduction

Burbot (*Lota lota*) gains attention in European intensive coldwater aquaculture due to its delicate white fillets, high nutritional value comparable to its relative, Atlantic cod, and valuable by-products such as liver, roe, and skin. Burbot exhibits environmental requirements similar to those of trout and achieves comparable growth rates, while demonstrating greater hardiness and lower disease susceptibility than other salmonids. These characteristics position burbot as a promising candidate for diversifying inland freshwater aquaculture (Oliver *et al.*, 2024). Since 2012, commercial hatcheries have emerged in Belgium and Germany and fingerling availability is no longer a bottleneck. Although burbot aquaculture remains in its early stages, Oliver *et al.* (2024) indicate that culture methods are now adequate for commercial production. However, no studies have been conducted on the longterm effects of feeding levels on growth of juvenile burbot weighing over 30 g (Trejchel *et al.*, 2014).

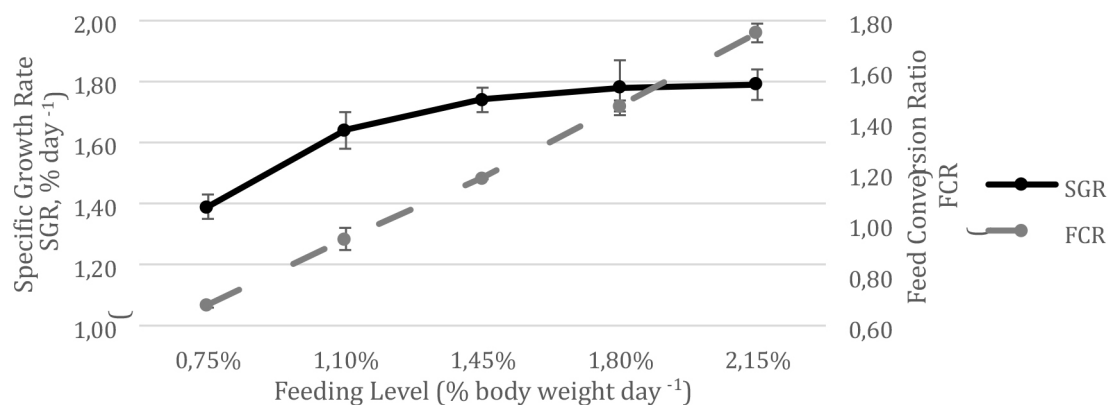
The aim of this study is to determine the effect of feeding levels on growth, condition and body composition of growing juvenile to subadult burbot in RAS to identify the true optimal feeding level.

Materials and methods

In 15 cylindrical 120 L tanks, 60 fish were stocked per tank, of which 32 were individually PIT-tagged. The fish had an average individual body weight of 34.66 ± 7.24 g. All tanks were connected to a single intensive RAS, and the temperature was maintained at 15 °C. Five treatments, each in triplicate, were assigned feeding levels ranging from 0.75 to 2.15% of biomass per day. Fish were fed a commercial diet (Protec, 3 mm, Skretting) using automatic feeders.

Every three weeks, all fish were sedated and sampled. Of each PIT-tagged fish, body weight and length were recorded. Specific growth rate (SGR), thermal growth coefficient (TGC) and Fulton's condition factor (K) were calculated. Total biomass per tank was measured to determine daily feed rations for each treatment and to calculate the biological feed conversion ratio (FCR_b). After the final sampling in week 15, whole-body proximate composition was determined for six randomly selected pooled fish per tank. In addition, the viscerosomatic index (VSI), hepatosomatic index (HSI), and Carcass Yield were measured in eight randomly selected individual fish per tank.

Figure 1: Effect of feeding level on specific growth rate and feed conversion ratio



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Table 1: Growth and feed utilization metrics under different feeding levels

Parameter	Feeding level (% BW day ⁻¹)				
	0.75%	1.10%	1.45%	1.80%	2.15%
Individual body weight W0 (g)	34.58 ± 7.48 ^a	34.22 ± 7.06 ^a	34.83 ± 6.28 ^a	34.63 ± 7.45 ^a	34.56 ± 8.13 ^a
Individual body weight W15 (g)	92.62 ± 23.71 ^a	109.40 ± 30.07 ^b	118.69 ± 26.25 ^c	122.61 ± 33.37 ^c	123.21 ± 32.53 ^c
Initial condition factor W0	0.61 ± 0.07 ^a	0.60 ± 0.05 ^a	0.60 ± 0.04 ^a	0.60 ± 0.06 ^a	0.61 ± 0.05 ^a
Final condition factor W15	0.62 ± 0.06 ^a	0.65 ± 0.07 ^{a,b}	0.65 ± 0.07 ^b	0.66 ± 0.07 ^b	0.67 ± 0.07 ^b
Stocking density W0 (kg m ⁻³)	17.23 ± 0.18 ^a	17.07 ± 0.10 ^a	17.30 ± 0.14 ^a	17.09 ± 0.06 ^a	17.20 ± 0.02 ^a
Stocking density W15 (kg m ⁻³)	44.34 ± 0.20 ^a	51.71 ± 2.41 ^b	58.02 ± 2.46 ^c	56.43 ± 0.31 ^c	58.56 ± 1.12 ^c
SGR W0-W15 (% day ⁻¹)	1.39 ± 0.04 ^a	1.64 ± 0.06 ^{a,b}	1.73 ± 0.04 ^b	1.78 ± 0.09 ^b	1.79 ± 0.05 ^b
TGC W0-W15	0.78 ± 0.02 ^a	0.95 ± 0.05 ^{a,b}	1.04 ± 0.04 ^b	1.07 ± 0.03 ^b	1.08 ± 0.05 ^b
FCR _b W0-W15	0.68 ± 0.01 ^a	0.94 ± 0.04 ^b	1.18 ± 0.00 ^c	1.46 ± 0.02 ^d	1.75 ± 0.04 ^e

All data are expressed as mean ± SD. Results were analyzed using a linear mixed-effects model, followed by Tukey's post hoc test. Values marked with different letters show significant differences ($P < 0.05$).

Results and discussion

SGR and TGC increase with feeding level but level off from 1.45% feeding level, while FCR_b steadily increases with further increasing feeding level. Therefore, the optimal feeding level is around 1.45% body weight per day, where SGR is near maximal and FCR_b remains relatively efficient. A feeding level of 1.10% will result in a more efficient FCR_b with only a minor reduction in growth (Figure 1). Fulton's condition factor (K) was lower in only the 0.75% feed level treatment (Table 1). Hepatosomatic Index (HSI) was significantly higher in the 1.45%, 1.80% and 2.15% feeding level treatments compared to 0.75%, while no statistic differences could be shown for Carcass Yield (data not shown).

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CAN PROTEIN HYDROLYSATES IMPROVE PERFORMANCE AND GUT HEALTH UNDER FLUCTUATING TEMPERATURES IN GILTHEAD SEABREAM?

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Introduction

Gilthead seabream (*Sparus aurata*) is a key species in European aquaculture; however, its production is challenged by low-temperature stress, impairing growth, metabolism, and immune function. Increasing thermal variability in Atlantic waters is expected to intensify these challenges by subjecting fish to repeated temperature fluctuations that disrupt homeostasis and limit recovery between stress events. Functional diets enriched with protein hydrolysates from animal by-products are emerging as a strategy to enhance fish resilience under stress by providing bioactive peptides and immunostimulants. However, their effectiveness under fluctuating temperature conditions remains unclear. Therefore, this study aimed to evaluate whether dietary inclusion of poultry or swine protein hydrolysates can mitigate the impacts of fluctuating temperature on growth, nutrient utilisation, and intestinal health in gilthead seabream.

Material and Methods

Juvenile gilthead seabream (~23.8 g) were fed three isoproteic and isoenergetic diets: with 3% of commercial fish hydrolysate (CSPS90; COM), 3% poultry hydrolysate (POULTRY) or 3% swine hydrolysate (SWINE). Fish were fed three times per day to apparent satiety and maintained under constant (CT; 22 °C) or fluctuating temperature (FT; 22-15-22 °C) regimes in a recirculating aquaculture system. Growth and feed intake were monitored every 4 weeks at three time points: before, after prolonged exposure, and post-recovery. At the end of the trial, nutrient utilisation and anterior intestinal morphology were also assessed. Nutrient and energy apparent digestibility coefficients were determined in a parallel trial, at 15 °C and 22 °C.

Results

Thermal regime influenced all measured parameters, with FT reducing body weight, voluntary feed intake, nutrient utilisation, and whole-body composition. Distinct dietary effects were observed despite the influence of temperature. All dietary treatments supported growth, with a trend toward increased performance in the POULTRY group. Differences in voluntary feed intake were observed between diets, with higher values in the POULTRY diet compared to the SWINE diet, alongside differences in whole-body lipid content. Nutrient and energy digestibility was higher at 15 °C than at 22 °C and was not significantly affected by diet. While FT did not affect anterior intestine integrity, it significantly reduced the number of acid and total goblet cells; whereas the POULTRY diet increased goblet cell numbers.

Discussion

The negative effects of fluctuating temperature on performance are consistent with reduced feed intake under thermal stress. The higher feed intake observed with the POULTRY diet may be indicative of differences in palatability, likely related to the presence of low-molecular-weight peptides. This higher feed intake could be linked to the differences in lipid deposition and growth trends. In contrast, the SWINE diet maintained growth despite lower feed intake, indicating differences in nutrient utilisation. The reduction in goblet cell numbers under FT indicates impaired mucosal function, which was partially mitigated by the POULTRY diet. Digestibility was higher at lower temperatures, likely resulting from reduced feed intake at lower temperatures and longer gastrointestinal transit time, which can enhance nutrient absorption efficiency. However, digestibility was not affected by diet, suggesting that absorptive capacity was not directly linked to differences in goblet cell abundance.

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MANIPULATING THE DEGRADOME OF *Nephrops norvegicus* USING BACTERIOPHAGE

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The Norway lobster (*Nephrops norvegicus*), also known as langoustine, is economically the most important crustacean in the UK, with an annual value reaching £100 million. However, harvested langoustines spoil quickly, typically remaining fresh for only 3 to 5 days. This is caused by specific spoilage organisms (SSOs) – naturally occurring, non-harmful bacteria that grow rapidly in seafood. The accumulation of bacterial metabolites leads to unpleasant odours, resulting in ~30% of the still-edible product being rejected by the customers and discarded as a waste.

Bacteriophages (commonly known as phages) are viruses that specifically infect and kill bacteria. They are the most abundant organisms on Earth and are easily isolated from the environments where their bacterial hosts are found. Due to their high specificity and effectiveness, they are ideal candidates for biocontrol of the bacteria in sectors where antibiotic use is restricted, such as the food and seafood industry.

In this project, we identified how the microbiome changes during degradation within a 16 day period (Elsheshtawy et al., 2026). We then isolated a range of phages that specifically target the dominant SSOs responsible for spoilage in freshly caught langoustines. Subsequently, we assessed their impact on the microbiome and the formation of the malodour causing metabolites such as trimethylamine in a 14-day degradation trial. Our research demonstrated that phages can be used to manipulate the microbiome and alter the rate at which the growth of bacteria that generate undesirable smells during the storage of langoustines occurs.

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QUANTIFYING HARVESTABLE BIOMASS AS AN INDICATOR OF MICRO-SITE SUITABILITY FOR LOW-TROPHIC AQUACULTURE IN MULTI-USE WITH OFFSHORE WIND

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Introduction

In just four years' time global demand for seafood will exceed supply by 40 million metric tonnes yet aquaculture production in Europe has stagnated, largely due to competition for marine space. Maximising spatial efficiency and sustainable development across competing Blue Economy sectors is fundamental to meeting future aquaculture needs, and thus the Sustainable Development Goals of the United Nations Agenda 2030. In Europe, offshore wind farms (OWF) dominate ocean space and present an ideal candidate for 'multi-use' with aquaculture. However, the open ocean remains largely untapped as a farming resource and consequently production potential within OWFs remains unclear. To encourage investment in and future development of multi-use of OWF and low-trophic aquaculture (LTA), scenario analyses that give an indication of site suitability, production potential, and therefore economic feasibility, are critical.

Methods

Here, we used a coupled 3D hydrodynamic-biogeochemical-sediment model combined with dynamic energy budget/growth models for two key LTA species – *Mytilus edulis* and *Saccharina latissima* – to assess harvest potential within Kriegers Flak OWF in the southern Baltic Sea. We first assessed the spatial variability of 'harvest potential' of the two species within the OWF based on the food and nutrient fluxes to the area and the maximum biomass yield given no depletion of food or nutrients. We used these metrics to assign 'high', 'medium', and 'low' harvest potential areas to both species. Using these harvest potential categories we then explored optimum farming scenarios (see Figure 1 for example) to quantify maximum harvestable biomass of *M. edulis* and *S. latissima* within the OWF area.

Results

We found significantly greater biomass for *M. edulis* farms located in the high harvest potential areas compared to the medium or low harvest potential areas. Equally, harvestable biomass of *M. edulis* significantly increased with increasing distance between farm positions, indicating a strong effect of depletion on growth. On the other hand, low salinities in the southern Baltic Sea strongly inhibited the growth of *S. latissima*, as evidenced by the results of a sensitivity analysis, and consequently no such patterns were evident for this species. Rather, harvestable biomass for *S. latissima* remained very low and non-profitable across all model scenarios. Additionally, we found little spatial overlap in high harvest potential areas for *M. edulis* and *S. latissima*, suggesting the potential benefits of co-culture of the two species in this area is limited.

Discussion

This study represents a significant advancement in spatial planning for OWF-LTA multi-use. The significant difference in *M. edulis* biomass between model scenarios suggests farm placement is crucial to ensuring the economic viability of LTA within OWF, and highlights the need for predictive models to enhance LTA production at the planning stage of multi-use. Our results support future micro-siting decisions of LTA within the Kriegers Flak OWF and, more broadly, outline a methodological framework for assessing harvest potential of LTA species using ecosystem models. This framework can be applied globally to increase the evidence-base for the viability of multi-use of OWF and LTA Blue Economy sectors.

Acknowledgment

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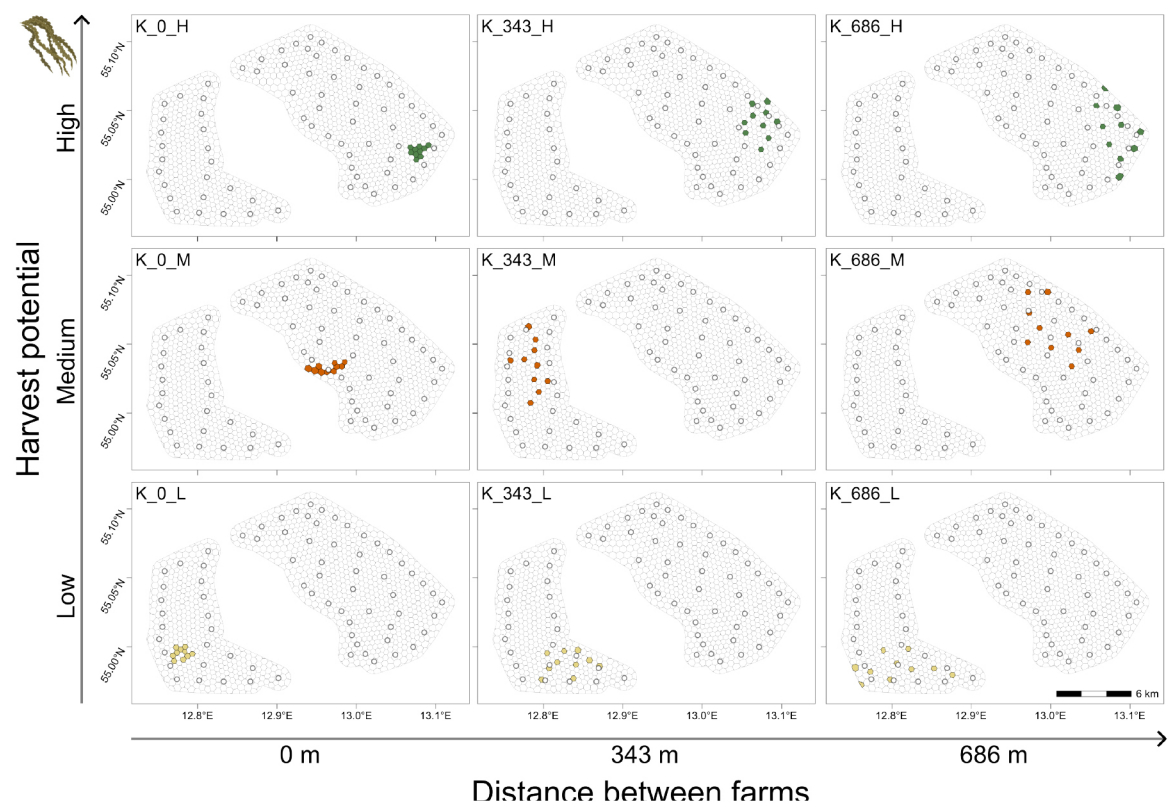


Figure 1. Farm locations under the nine model scenarios used to assess harvestable biomass potential of kelp in the Kriegers Flak offshore wind farm. Ten farms were randomly placed per scenario. Farm positions are indicated by coloured polygons. The x axis indicates increasing minimum distance between farms. The y axis indicates the harvest potential category of the area in which the farms were placed.

MICRO-SITING TOOL FOR PLANNING LOW-TROPHIC AQUACULTURE NEAR OFFSHORE WIND FARMS

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Offshore Wind Farms (OWFs) present promising opportunities for multi-use co-location with Low Trophic species Aquaculture (LTA) in the North Sea and Baltic Sea, leveraging shallow coastal areas and existing infrastructure. Because LTA systems do not require external feeding, this integrated approach significantly reduces expenditure on feed production and marine logistics. Furthermore, co-location offers environmental benefits; in the eutrophic Baltic Sea, farming and harvesting LTA can mitigate excess nutrients, thereby improving localized water quality and supporting marine restoration.

In the context of the Horizon Europe OLAMUR (Offshore Low-trophic Aquaculture in Multi-Use scenario Realisation) project, we developed a Python tool which has also been integrated into the interactive web interface Operational Decision Support System <https://gis.sea.ee/odss/mstool/model>. This tool allows users to choose a location to analyze fuel cost, monthly nutrient intake, and biomass yields for blue mussel (*Mytilus edulis*) and sugar kelp (*Saccharina latissima*) at a high resolution of 200 m x 200 m. Currently optimized for the Kriegers Flak OWF in Denmark, expansions are underway to integrate the Helgoland OWF in Germany.

A key feature of the tool is its ability to model blue mussel intra-farm interactions, assessing potential nutrient depletion and subsequent biomass reduction caused by adjacent farm installations (Figure 1). This capability enables stakeholders to plan multiple farms while mitigating biomass loss.

Since 2003, the European Union has recognized Life Cycle Assessment (LCA) as the preferred framework for evaluating environmental impacts. The micro-siting tool and associated website will incorporate the total environmental footprint of sugar kelp for each cultivation site (Sains de la Maza Larrea, 2026). This integration will enable users to verify whether a given farm, when its own environmental impacts are accounted for, yields a true net environmental gain.

Acknowledgment

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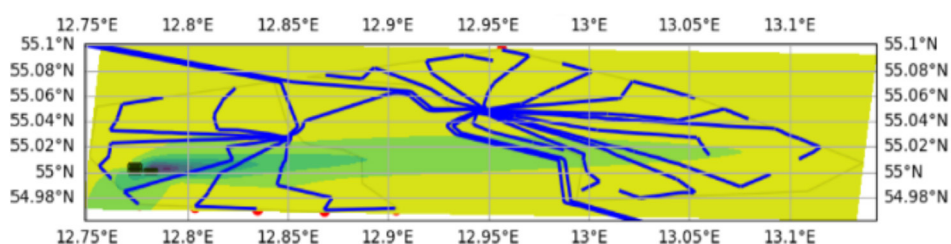


Figure 1: Potential shadowing effect between blue mussel farms. In green, the shadowing by test farms at other potential farm locations in the wind park. In blue, the restricted cable zone inside the Kriegers Flak.

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 POLYRAS¹ 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, aims 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 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, reduce feed waste, and support species welfare. Results on growth performances and preliminary results on fish welfare are presented in this study.

Material and Methods

The experiment took place over a 174-days experimental period in seven 1 m³ tanks in a RAS supplied with water at 23 °C under a 12L:12D photoperiod. Three experimental rearing conditions were tested: pikeperch monoculture, European catfish monoculture, and biculture 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 167 g for pikeperch and 182 g for catfish. Pikeperch were fed with floating pellets (Le Gouessant Percid Grower®, 52% proteins, 15% lipids) to satiation, 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 at the end of the experiment. Fin condition in pikeperch, as well as fin and barbel condition in European catfish, were evaluated (adapted from Policar et al., 2016) to assess fish welfare.

After a normality and homogeneity test of variances (Shapiro–Wilk and Bartlett tests), an ANOVA with Tukey's post-hoc or Kruskal–Wallis with pairwise Wilcoxon tests were applied as required ($P < 0.05$).

Results

Survival rates were up to 95% for all batches. Final mean body weights were similar between mono- and polyculture for pikeperch (respectively 612 ± 152 g ; CV=24.1% and 602 ± 145 g ; CV= 23.8%) and catfish (respectively 1186 ± 303 g ; CV = 25.1% and 1218 ± 335 g ; CV = 27.1%) with no significant differences ($P > 0.05$). Specific growth rates (SGR) were similar between mono- and polyculture for pikeperch (respectively 0.71 and 0.72) and catfish (respectively 1.05 and 1.08). Food conversion ratio (FCR) in polyculture (0.87) was intermediate between those observed in pikeperch monocultures (1.01) and catfish monoculture (0.82). Period-specific SGR analysis highlighted higher growth in polyculture under specific conditions.

After the first three months of experiment, pikeperch reared in monoculture exhibited significantly better fin condition than those in polyculture ($P < 0.05$), but this difference was no longer observed at the end of the experiment. In contrast, no difference in fin and barbel condition was detected for catfish after three months, whereas at the end of the experiment, individuals reared in polyculture showed significantly better condition than those in monoculture ($P < 0.05$).

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Conclusions and perspectives

Our results indicate that pikeperch–catfish biculture in RAS can sustain favorable growth performances without compromising survival rate and welfare of both species. To further evaluate the potential of the system, isotopic analyses are ongoing to identify the source of the ingested feed and confirm the hypothesis of the valorisation of uneaten feed by catfish. Additionally, cortisol levels in pikeperch scales will be measured to monitor physiological stress (adapted from Carbajal et al., 2018). Based on our observations, we suggest that the performance of this biculture may be enhanced through implementation of both cohort management and increased initial stocking density. Feed valorization and a comprehensive assessment of fish welfare will be addressed in future work based on the present results.

Acknowledgment

This work was funded by Interreg Great Region and European Regional Development Fund (ERDF).

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- Optimization of aquaculture production in RAS (recirculating aquaculture systems) and reduction of its ecological footprint through polyculture in the Greater Region

EUROPEAN SEABASS AND GILTHEAD SEABREAM REQUIRE TAILORED MICRODIETS FOR FIRST FEEDING

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Introduction and Aim

Early larval nutrition remains one of the main bottlenecks in marine fish aquaculture, strongly influencing survival, growth, robustness and juvenile quality. Recent advances in inert microdiet technology aim to reduce reliance on live feeds while improving nutritional precision and sustainability, including the use of novel ingredients such as algae-derived components and protein hydrolysates. This work synthesises results from four controlled feeding trials evaluating innovative microdiets during first-feeding and early weaning of two important Mediterranean aquaculture species: gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*). The overall aim was to assess how dietary formulation and feed processing innovations can enhance larval performance, thereby supporting the development of effective alternatives to live prey in hatcheries.

Experimental design

Trials were performed at IPMA (Portugal) facilities. Larvae were reared in 300 L cylindro-conical tanks (open circulation, 36‰ salinity) under controlled conditions. Seabass were kept at 16.5 ± 1 °C and offered experimental microdiets starting at 8 or 15 days after hatching (DAH) (depending on the trial) through to 50 DAH, under a co-feeding regime with live prey. Seabream were kept at 18 ± 1 °C, with microdiets introduced at 2 or 3 DAH (depending on trial) through to 50 DAH, also in co-feeding with live feed. Dietary treatments in these trials targeted variations in feed formulation — such as peptide hydrolysis level (producing different average peptide sizes), inclusion level of marine protein ingredients, and inclusion of algal fractions — as well as feed processing technology (fluidized bed agglomeration vs. micro-extrusion). Each trial monitored key performance indicators including larval growth (length and dry weight), survival, and incidence of skeletal deformities.

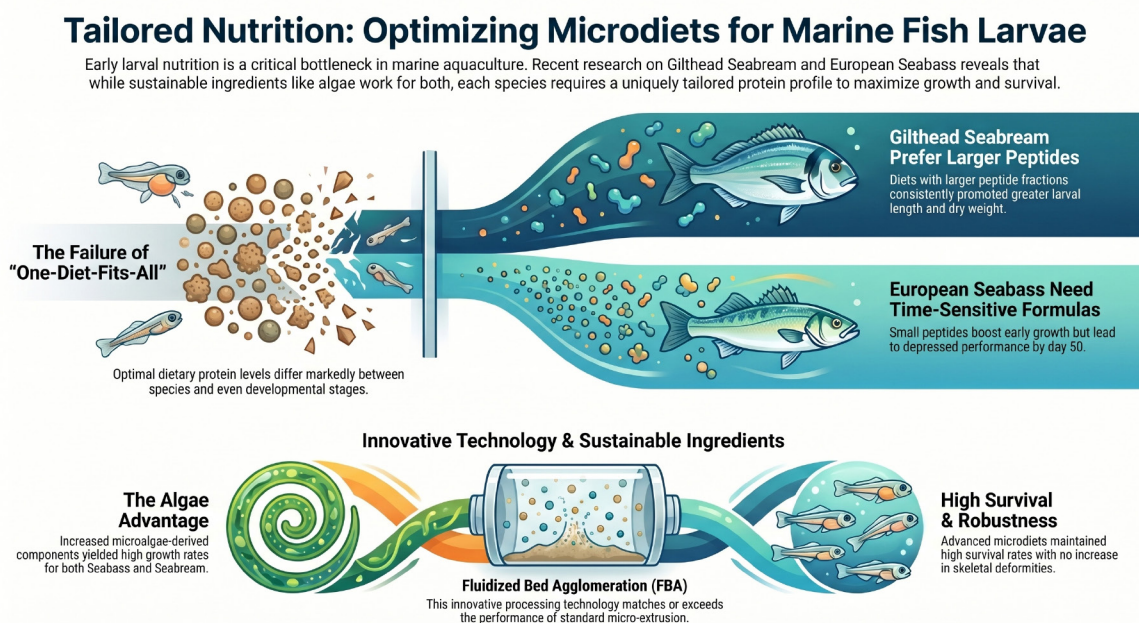


Figure 1. Infographic summarizing Algae Vertical findings on how dietary formulation and feed processing innovations can enhance first feeding larval performance.

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Results and Discussion

Across all trials and for both species, the novel microdiets produced by fluidized bed agglomeration (FBA) supported good larval performance, in some cases matching or even exceeding the growth achieved with reference diets produced by standard micro-extrusion. No significant differences in survival or in the incidence of skeletal deformities were observed among dietary treatments within each trial. However, clear species-specific responses to diet were evident. In seabream larvae, diets formulated with larger peptide fractions consistently promoted greater larval length and dry weight compared to diets with more extensively hydrolysed (smaller) peptides. In contrast, seabass larvae showed an opposite trend: at an early stage (22 DAH), a diet containing smaller peptides resulted in improved growth relative to the control, but by the end of the nursery phase (50 DAH) this same diet led to depressed growth performance.

Conclusions

The optimal level and type of marine protein ingredients for maximizing growth differed between gilthead seabream and European seabass. Nonetheless, increased inclusion of algal ingredients (such as microalgae-derived components) yielded good growth in both seabream and seabass, indicating the potential of these sustainable ingredients in larval diets. Overall, these results demonstrate major progress in first-feeding strategies for marine fish, driven by advances in microdiet formulation and production technology and by the use of alternative ingredients (including algae). Crucially, the data show that optimal diet characteristics differ markedly between fish species, and even between developmental stages within a species, indicating that a “one-diet-fits-all” approach is not appropriate. Tailored, species-specific microdiets are therefore essential to maximize larval performance and quality in order to support aquaculture sustainability.

Acknowledgments

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NUTRITION DRIVES VIABILITY AND ROBUSTNESS OF WHITELEG SHRIMP POST-LARVAE BEYOND GROWTH RESPONSES

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Introduction and Aim

Reliable nursery feeds for whiteleg shrimp (*Penaeus vannamei*) post-larvae need to do more than support fast biomass gain: they must also sustain survival, efficient feed use and the capacity to withstand abrupt environmental challenges. In clear-water nursery systems, where formulated microdiets provide the main nutritional input, small changes in marine ingredient profile, lipid supply or functional supplementation may therefore have consequences that are not fully visible when only final weight is considered. This study combines two controlled feeding studies designed to clarify how microdiet composition affects post-larval performance, physiological condition and robustness. The overall aim was to determine whether marine protein source, lipid level and selected functional ingredients can improve the quality of shrimp post-larvae for hatchery and nursery production.

Experimental design

Both trials were conducted under clear-water experimental nursery conditions using inert microdiets produced at Sparos Lda (Olhão, Portugal) and shrimp rearing at Riasearch Lda (Murtosa, Portugal). In the first study, five microdiets were compared over 21 days: a formulation based on a fish and squid meal blend, three diets dominated by fish meal, squid meal or krill meal, and a fish-meal diet with higher lipid inclusion. The main diet set was formulated around similar protein and moderate lipid contents, while the high-lipid fish-meal diet increased lipid inclusion from approximately 14% to 16%. Growth, relative growth rate, feed conversion ratio, survival, whole-body composition, oxidative stress biomarkers, immune parameters and the expression of antioxidant- and immune-related genes were evaluated. In the second study, shrimp were fed from 19 to 40 days after hatching with four selected treatments: CTRL-, CTRL+, SAFG and ULCTP4. These represented, respectively, a basal diet, a high-quality marine-based formulation, the later formula supplemented with beta-glucan, and the same base supplemented with broken-cell microalgae plus seaweed biomass. Weight, relative growth rate, survival, feed intake and feed conversion ratio were assessed, followed by a salinity-stress challenge to evaluate post-larval resilience.

Results and Discussion

The two datasets converged on a central message: growth was comparatively stable across diets, whereas survival, feed efficiency and condition-related indicators were more responsive to formulation. In the marine ingredient trial, final wet weight and relative growth rate did not differ significantly among treatments, with an overall mean final weight of 255 ± 26 mg and relative growth rate of $15.1 \pm 0.5\%$ day⁻¹. However, survival and feed conversion separated the diets. The fish-squid blend and krill meal formulations reached survival values close to 80%, while the high-lipid fish-meal formulation showed the lowest survival ($41.5 \pm 15.0\%$) and a higher feed conversion ratio than the krill meal diet. Single-source marine protein diets did not provide the same overall profile as the blended marine protein or krill-based diets, indicating that ingredient quality and complementarity can be more relevant than growth alone suggests.

Physiological and molecular measurements helped explain these differences. Shrimp fed the high-lipid fish-meal diet showed higher catalase, glutathione S-transferase and pro-phenoloxidase activities than shrimp fed the krill meal diet, indicating stronger activation of antioxidant and immune pathways. Gene-expression patterns also separated the fish, squid and high-lipid fish-meal diets from the fish-squid blend and krill meal groups. In particular, multivariate analysis of transcripts associated with antioxidant defence and immune condition discriminated the high-lipid and the fish meal treatments, from other formulations, supporting that excess lipid inclusion increased physiological cost without improving growth, and fish meal should not be used in as dominant ingredient.

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The functional-ingredient trial reinforced the message that growth stays stable across diets, but PL robustness does not. Final body weight and relative growth rate were not statistically different among these four diets. Nevertheless, the basal CTRL- diet had the lowest survival ($13.6 \pm 1.15\%$) and the poorest feed conversion ratio (1.63 ± 0.25), together with the highest feed intake (0.59 ± 0.05 g shrimp⁻¹). CTRL+, SAFG and ULCTP4 all improved survival relative to CTRL-, reaching $21.6 \pm 1.29\%$, $24.9 \pm 3.44\%$ and $23.6 \pm 11.37\%$, respectively. Feed conversion also improved in the same treatments, with values of 0.88 ± 0.05 for CTRL+, 0.85 ± 0.04 for SAFG and 0.97 ± 0.08 for ULCTP4. During the salinity challenge, SAFG showed the strongest survival probability among the selected treatments, indicating that beta-glucan supplementation enhanced robustness even when delivered in a high-quality marine-based formulation.

Conclusions

These studies show that similar growth can mask substantial differences in post-larval quality. Survival, feed conversion, resilience to a salinity challenge, antioxidant and immune biomarkers, and gene-expression patterns all provided information that was not captured by final weight or relative growth rate alone. The use of complementary marine protein sources or krill meal improved shrimp post-larval performance and health status under the tested clear-water nursery conditions. Increasing dietary lipid above approximately 14% in a fish-meal formulation did not enhance growth and was associated with poorer survival and higher physiological activation. Finally, beta-glucan supplementation improved robustness, including challenge resilience, even when added to a high-quality marine-based diet. Overall, diet evaluation for shrimp post-larvae should combine zootechnical, challenge and health-related indicators, because a one-parameter assessment based only on growth is insufficient for developing microdiets that deliver viable and resilient animals.

Acknowledgments

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CIRCULAR AQUACULTURE: VALORIZATION OF CHITIN-RICH BY-PRODUCTS FROM INSECT REARING AS AN AQUAFEED INGREDIENT FOR RAINBOW TROUT *Oncorhynchus mykiss* CULTURE

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Introduction

Aquaculture represents the fastest-growing sector of animal production. However, its continued growth and long-term viability depends on developing innovative and sustainable aquafeed formulations. Chitin has recently emerged as a promising additive in aquafeeds, due to its potential to improve growth, intestinal health, and immunity of aquatic organisms. In fish, chitin can be degraded by endogenous enzymes (chitinases). However its metabolic response is dose- and particle size-dependent. At appropriate levels, chitin exhibits immunostimulatory properties and improve gut health. Reported effects include increased goblet cell density, enhanced mucus production, and strengthened intestinal barrier. At the molecular level, it modulates the expression of immune-related genes, including pro- and anti-inflammatory cytokines such as *il1 β* , *tnfa*, and *il10*.

Traditionally derived from shrimp and yeast, chitin can also be obtained from insects, which are considered a promising protein source for animal nutrition. Notably, insect farming generates by-products such as pupal exuviae and the exoskeletons of deceased adults, which are rich in chitin. This presents an opportunity to develop value-added feed ingredients. Rainbow trout (*Oncorhynchus mykiss*) is the most extensively farmed freshwater species in Europe, and efforts to improve its sustainable production remain a central research focus.

Within this framework, the ACInA project integrates aquaculture, insect farming, and agriculture into a circular-economy model. The strategy aims to improve sustainability by valorizing waste and enhancing overall resource efficiency. Insects, reared on substrates derived from agri-food by-products, enable their conversion into high-value biomass. Insects' derivatives are used to formulate functional aquafeeds, while aquaculture waste are converted into fertilizers for agricultural use.

In this context, the present study aimed to extract chitin from insects and incorporate it as functional additive into diets for Rainbow trout, to evaluate its effects on fish growth performance and overall physiological responses.

Materials and Methods

Exuviae collection. Agricultural organic by-products were used as rearing substrate for *Hermetia illucens* in a vertical farming system (Baofly S.r.l., Trento, Italy). Insect larvae were reared at 27–28 °C, 70% humidity, and in darkness. At the end of the cycle, exuviae and dead adults were collected, dried, and milled. The recovered products were used to formulate experimental trout diets.

Experimental diets. Starting from a commercial diet specific for rainbow trout (VRM S.r.l., Trento, Italy), which was used as Control diet (C), five experimental diets were obtained: (i) C+ diet, (control diet + 3% w/w chitin); (ii) IM5 diet, (control diet + 3% w/w chitin + 5% insect meal); (iii) IM7 diet, (control diet + 3% w/w chitin + 7% insect meal); (iv) IM10 diet, (control diet + 3% w/w chitin + 10% insect meal).

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Experimental design. Three hundred and seventy-five rainbow trout (initial body weight: 200-220g), were initially acclimatized for two weeks at the experimental fish facility Fondazione Edmund Mach; Centro di Trasferimento Tecnologico, San Michele a./A., Italy) and then divided into 12 open circulating tanks (800L; three tanks per experimental group), obtaining five experimental groups. Fish were hand-fed the experimental diets 5 days per week from 2% to 1.5% of the biomass adjusting it by measuring the 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) were weighted and zootechnical parameters were measured. Samples from liver, intestine and stomach were obtained and properly stored for subsequent analyses.

Analyses. Biometric and zootechnical indexes: (i) survival rate, (ii) growth performances calculating the specific growth rate (SGR%), and weight gain (WG); (iii) feed conversion ratio (FCR), to evaluate feed efficiency; histological analyses of the liver to assess parenchymal architecture and lipid deposition, and of the distal intestine to evaluate histopathological indices and tissue integrity; molecular expression of genes involved in immune response (*il1b*, *il10*, *tnfa*) and on chitin digestion (*chia*) assessed in distal intestine and stomach, respectively.

Results

Survival was 100% across all experimental groups while no significant differences were observed in zootechnical performance, including WG, SGR and FCR. Liver histology showed a generally normal parenchymal structure in all groups. However, the degree of lipid accumulation varied among treatments. In the distal intestine, no significant differences were detected in the evaluated histopathological indices while supplemented diets affected the density of goblet cells and of supranuclear vacuoles. Considering molecular analysis, dietary treatments modulated immune-related gene expression, influencing the expression of inflammatory markers. Genes related to digestive or metabolic functions were also influenced in response to specific dietary components.

Discussion

Overall, fish maintained optimal health and growth across all dietary treatments, with no adverse effects on general welfare. Histological analyses confirmed normal tissue structure in key metabolic organs, indicating good physiological tolerance to the tested diets.

Dietary supplementation with insect meal proved to be an effective strategy, supporting positive physiological responses in fish. The moderate increase in hepatic lipid accumulation observed was not associated with pathological conditions and is likely related to the fatty acid profile of the ingredients. Differences in goblet cell abundance appears to reflect an adaptive response to chitin, a polysaccharide that can influence gut function by stimulating mucus production.

Chitin may also act as a natural immunostimulant, contributing to enhanced immune efficiency. Therefore, optimizing its inclusion level and physicochemical properties is crucial to improving the nutritional value and functional benefits of insect-based diets, while ensuring fish health and promoting more sustainable aquaculture practices.

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REGULATING PATHWAYS OF TOLERANCE TO EXTREME HEAT AND SALINITY IN OYSTERS: CASE-STUDY ON THE QATARI PEARL OYSTER *Pinctada radiata*

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Introduction

Climate change represents an unprecedented threat to marine ecosystems, severely impacting the survival and distribution of marine species worldwide. As climate change accelerates, understanding how marine species adapt to environmental stressors has become a critical focus in marine biology. Extreme environments such as the Arabian Gulf can serve as natural analogues for future coastal conditions under global change. Here we studied the pearl oyster *Pinctada radiata* living in extreme environmental conditions along the Qatari coast line.

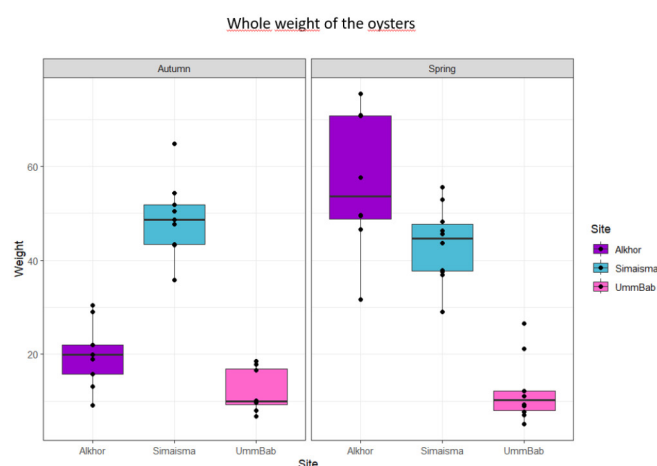
Environmental Proteomics.

The environmental proteomic approach using nano-LC MS MS is a powerful tool to understand regulating pathways of adaptation to stress in marine species (Tomanek, 2014; Guo et al., 2021; Gajahin Gamage et al., 2022). We analyzed *P. radiata* oyster natural populations from three field stations with contrasted ecological parameters, during two seasons. We used a global proteomic approach using Nano Light Chromatography tandem Mass Spectrometry (nano LC MS MS) to identify regulating pathways of oyster adaptation to extreme environmental temperature and salinity. We analyzed 1 million of hemocytes cells from each oyster, cells being freeze dried for their transfer in our laboratory before total protein extraction. We were able to identify 4,437 unique proteins in each individual sample of cells.

Results and Discussion.

For the first time we were able to analyse proteomics in cells from oyster sampled in their natural habitat, to identify individual mechanisms of adaptation in each population. We identified that 25% of the cell proteome in oyster hemocytes was regulated by the season ($p < 0.001$). We demonstrated that pathways for biomineralization, immunity, response to thermal and oxidative stress, as well as DNA replication, were modified between spring and summer. In the sampling site UmmBab, the oysters *P. radiata* are adapted to extreme salinity stress, surpassing 55 ppt, and exhibited strong size reduction (Figure 1), consistent with environmentally driven dwarfism, as recorded previously (Giraldes et al. 2023; Giraldes et al. 2026). Going deeper in the statistical analysis of our proteomic individual datasets, we were able to identify 375 proteins in hemocytes ($p < 0.001$) whose expression was correlated to oyster weight. Under extreme salinity, we identified proteomic changes in pathways related to mitochondrial ATP synthesis, regulation of protein synthesis, and response to oxidative stress. Overall, our study identified major functional changes in cellular pathways combined with oyster dwarfism as key adaptive responses to extreme salinity stress.

Figure 1.



(Continued on next page)

Acknowledgment

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RATIONAL SCALING IN AQUACULTURE DEVELOPMENT: SCALING OUT, NOT ONLY UP

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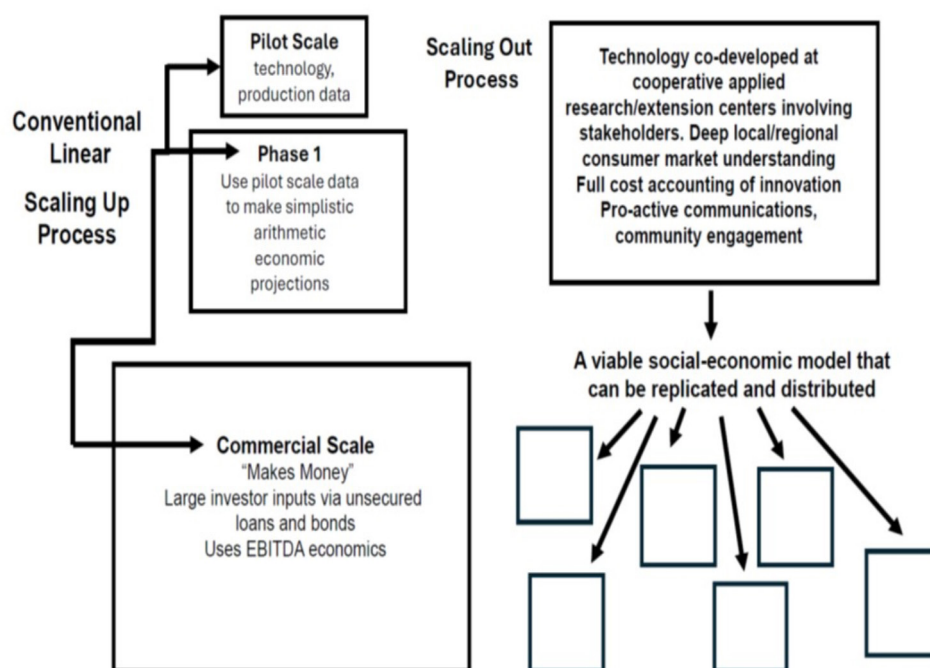
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Aquaculture facilities of high capital intensity and large demands for space have a risk of disconnection from communities, especially in rural areas. Here, scale becomes not merely technical and economic variables but social-political ones, as all the impacts of scaling are not comprehensively addressed. Scaling requires recognizing the complexities (time, different investment types, etc.) involved in facilitating a transition to a new normal, and aquaculture, as a “new” intervention in most of the world outside of Asia, requires systemic, not simplistic, scaling strategies. Overhyped scaling-up narratives can attract capital prematurely, ignore ecosystem and social limits, and undermine credibility if failures occur.

Conventional scaling up assumes that a successful pilot can be replicated elsewhere, that increasing size equals success, and that technical solutions can simply be transferred. Controlled, pilot project environments don’t always scale up. Pilot projects often rely on external funding, high-level technical, often-imported, leadership, and temporary incentives/subsidies. Such conditions do not represent real-world complexity. Many pilots fail because markets are not ready, governance is not aligned, and producers lose sustained incentives/subsidies. Smaller, economically viable aquaculture farms located in rural areas are more manageable, involve less risk, and produce less wastes, allowing integrated landscape designs for wastes as additional income sources in circular bioeconomies in contrast to mega-farms. Scaling up production without scaling value chains is unstable. Improper planning for scaling up can increase ecological impacts, concentrate economic power leading to even greater income disparities, marginalize small-scale producers, outpace governance capacity, and create social opposition. We suggest that aquaculture development not only consider scaling up growth in size or production output but also consider all scaling options to move toward strategies that embed ecological limits, social legitimacy, participatory governance, capacity building and institutional learning as the core tenants of sectoral expansion.



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Scaling Out Strategies: Aquaculture Ecotourism

There is a path of rational scaling rarely considered in aquaculture development by governance systems or investors - *scaling out* - not only scaling up (Figure). Scaling out strategies emphasize partnerships for well developed models at scale, replication, institutional diffusion, stakeholder and governance alignment rather than simple size and production expansion. Scaling out requires inter-sectoral partnerships who develop economically viable, smaller scale aquaculture models that can be distributed widely over diversified ecologies and networks. Tourism contributes ~10% of global GNP. Aquaculture ecotourism is one practical way to add value to aquaculture without necessarily scaling production volume, as it has similar network diversity, attention to value addition, and branding. Aquaculture ecotourism is most compatible with experiential, nature-based tourism. Aquaculture ecotourism (farm visits, participatory monitoring, food experiences, place-based narratives) creates new interfaces among producers, communities, scientists, educators, policymakers, conservation NGOs, and consumers. Its main benefits are: (1) Income diversification and creation of local, mostly rural, jobs via farm tours, tastings, festivals, snorkeling/diving, and education experiences that diversify and create new revenue streams and employment that can buffer farmers from commodity price swings and create multiplier economies for communities; (2) Strengthened local food systems (shorter value chains) by creating “eat local” demands and premium pricing for traceable seafood, helping farmers and communities capture more value locally rather than exporting it. Culinary/food tourism strengthens inter-sector linkages, learning, and local economic development; (3) Increased social license and environmental stewardship in rural areas by making production visible and accessible, improving understanding and acceptance when farms are well-managed, and providing an educational and partnership platform for monitoring (citizen science), biodiversity, and conservation practices. Research has shown that increasing and diversifying stakeholder engagement is central to aquaculture’s social acceptability. Partnerships with local academia and technology providers to develop transparent, accessible monitoring platforms can turn farms into living labs and field classrooms, another way to scale out benefits beyond production.

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SCIENCE OUTREACH AS A TOOL TO IMPROVE PERCEPTIONS OF AQUACULTURE IN A CHANGING WORLD

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Introduction

Aquaculture is central to the future of sustainable food systems, yet its social acceptance has not fully kept pace with its scientific and technological development. Farmed aquatic foods are still sometimes perceived as less natural, less healthy, or less desirable than wild alternatives, while concerns around animal welfare, disease, food safety, environmental impacts, and production practices continue to influence public trust. In this context, science outreach has an important role in translating research results into clear, credible, and audience-specific messages that explain how innovation can improve welfare, resilience, sustainability, and the production of high-quality aquatic protein.

In a context of global change, increasing pressure on food production, limited natural resources, climate-related stressors, disease risks, and concerns about animal welfare, large research projects must do more than generate new knowledge. They must translate results into accessible, credible, and useful outputs for the audiences that can apply, support, or better understand those results.

Approach

The Horizon Europe project ‘Improving Green Innovation for the Blue Revolution: new tools and opportunities for a more sustainable animal farming (IGNITION)’ provides a case study of strategic science outreach to inform on the sustainable practices and improve the perceptions of aquaculture. The project focuses on animal welfare under climate change scenarios, biomarker discovery, functional feeds, vaccination strategies, genomic tools, non-invasive biosensors, and prediction models for improved health monitoring and production management in aquaculture. These research areas are strongly connected to the One Health concept, recognising the links between animal health, environmental sustainability, food systems, and human wellbeing.

IGNITION’s communication, dissemination, and exploitation materials were developed progressively, following the evolution of the project itself. At an early stage, general materials such as the project flyer and poster were produced to introduce the project as a whole, explain its objectives, and provide a clear overview for broad audiences, including key stakeholders such as researchers, students, industry practitioners, policy makers, civil society, and the general public. As results became available, the materials gained technical detail and were increasingly tailored to specific target audiences and dissemination contexts.

Infographics and videos were then developed as tools to bridge the gap between technical content and public outreach. These formats translate complex project methodologies, scientific concepts, and emerging results into accessible visual narratives. Infographics support the explanation of project methods and results in a simple manner for non-specialist audiences, particularly through social media channels such as Instagram, while also serving as supporting materials in scientific and industry-focused meetings and conferences where project results are presented to specialised audiences. Videos follow a similar logic: some are designed to communicate the project to broader audiences, while others support both communication and dissemination.

Webinars, podcasts, and industry-oriented fact sheets were developed for more technical audiences, including researchers, aquaculture producers, breeders, feed manufacturers, professionals working in aquatic animal health and welfare, nutrition profiles, and other blue economy stakeholders. These materials provide more detailed access to project knowledge, allowing scientific and technical audiences to engage with specific research themes, methods, and outputs. The fact sheets, in particular, translate project findings into concise and practical insights that can support uptake and informed decision-making by the aquaculture sector.

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Educational games were developed as a dedicated outreach tool for the general public, particularly younger audiences, families and schools. These materials introduce aquaculture, animal welfare, disease detection, and sustainable production through playful interaction, making them suitable for science days, school visits to partner facilities, public events, and informal learning environments. Their purpose is not only to explain scientific concepts, but also to improve ocean and aquaculture literacy, challenge misconceptions about farmed aquatic animals, and contribute to greater social acceptance of aquaculture as a sustainable food production system.

Relevance and expected impact

This audience-specific approach supports the communication, dissemination, and exploitation responsibilities associated with European Union-funded research, increasing science literacy and the likelihood that project results reach the communities that can use them. For industry stakeholders, targeted materials can help accelerate uptake of innovations related to functional feeds, bioactive compounds, selective breeding, welfare monitoring, and resilience to climate and disease pressures. For scientific audiences, visual and narrative materials can strengthen the presentation of results and support knowledge exchange across disciplines. For non-specialist audiences, accessible outreach can help address persistent misconceptions about farmed aquatic animals and improve understanding of how responsible aquaculture contributes to food security, sustainability, and animal health.

In this way, IGNITION also contributes to improving public understanding of aquaculture as a sustainable means of producing high-quality protein in a world facing population growth, overfishing, environmental limits, and climate change. By matching content, format, language, and dissemination channels to each audience, the project shows that science outreach should not be treated as an end-of-project visibility exercise, but as a strategic process that connects research outputs with societal understanding, sectoral uptake, and long-term trust in sustainable aquaculture.

Acknowledgment

This work was developed within the IGNITION project, co-funded by the European Union and the United Kingdom Research and Innovation under Grant Agreement No. 101084651.

FORTIFISH: IMPROVING WELFARE AND IMMUNITY DURING FISH EARLY-LIFE STAGES THROUGH FORTIFIED FEEDS

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Background

Early-life stages represent one of the most critical periods in fish development, during which exposure to environmental, husbandry and nutritional stressors can compromise welfare, growth performance and survival. Mitigating stress-related impacts during these stages is therefore a major challenge for both the aquaculture industry and the biomedical research sector that relies on zebrafish (*Danio rerio*) as a model organism.

Bioactive peptides constitute a promising class of functional ingredients capable of exerting regulatory effects beyond their nutritional value. In fish, these compounds have been associated with modulation of the hypothalamic-pituitary-interrenal (HPI) axis, antioxidant protection, enhancement of innate immune functions, improved intestinal health and greater capacity to cope with environmental and husbandry-related stressors. FORTIFISH investigates the application of selected peptide fractions as dietary supplements during critical developmental windows. By targeting physiological pathways linked to stress responsiveness, inflammation, oxidative balance and growth regulation, these functional feeds are expected to improve welfare, robustness and performance in both marine aquaculture species and zebrafish used for biomedical research.

FORTIFISH is an innovative R&D project focused on the development of functional fortified feeds designed to enhance health, welfare and stress resilience in European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*) and zebrafish (*Danio rerio*). Building upon the results obtained in the NOSTRESS project (ALG-01-0247-FEDER-047122), which demonstrated the capacity of selected bioactive peptides to reduce the impact of stressors and improve productive efficiency from the earliest developmental stages, FORTIFISH aims to translate this knowledge into novel nutritional solutions suitable for commercial application.

Methodology

FORTIFISH is led by SPAROS, a pioneering SME in aquaculture nutrition, in collaboration with ATLANTIKFISH, a Portuguese aquaculture company, and a multidisciplinary consortium comprising the Faculty of Medicine of the University of Lisbon (FMUL), Gulbenkian Institute for Molecular Medicine (GIMM), Interdisciplinary Centre of Marine and Environmental Research (CIIMAR), Portuguese Institute for the Sea and Atmosphere (IPMA), S2AQUA CoLAB, Champalimaud Foundation and the Institute for Research and Innovation in Health (i3S).

The project focuses on the formulation and validation of peptide-fortified diets tailored to larval and juvenile fish. Nutritional, physiological and molecular approaches are being integrated to evaluate their effects on stress responsiveness, health and performance. The resulting nutritional solutions are intended for industrial application in marine fish hatcheries and pre-growing systems, supporting improved welfare and more sustainable production practices. In parallel, the project will deliver innovative feeding strategies for zebrafish used in biomedical research.

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CIIMAR role in FORTIFISH is related to the study of immune-related biomarkers in fish early-stage juveniles and larvae, which will be complemented with physiological, oxidative stress and growth-related biomarkers by joining forces with S2AQUA and IPMA. Biomarkers will be analysed using enzymatic assays and real-time quantitative PCR, providing an integrated assessment of neuroendocrine, antioxidant and immune responses in fish subjected to experimental stress and pathogen challenges. This approach will enable the identification of dietary strategies that enhance stress resilience, health status and performance during early development.

Expected outcomes

By combining advanced functional nutrition with a strong industry-academia partnership, FORTIFISH aims to establish new standards for early-life-stage fish management, promoting resilience, welfare and productivity across both aquaculture and biomedical applications.

Acknowledgment

This work is part of FORTIFISH project, No. 21286, Operation No. MPr-2024-9-21286 (COMPETE2030-FEDER-02232500, LISBOA2030-FEDER-02232500, ALGARVE-FEDER-02232500), co-financed by the European Union through the Algarve 2030, Lisboa 2030 and COMPETE 2030 Operational Programs, in the framework of Portugal 2030.

IMMUNOMODULATORY EFFECTS OF ALGAL EXTRACTS AND IMMUNOSTIMULANTS ON EUROPEAN SEABASS RESISTANCE TO *Photobacterium damsela* subsp. *piscicida*

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Introduction and Aim

European seabass (*Dicentrarchus labrax*), an important mediterranean aquaculture species, is increasingly threatened by bacterial pathogens, including the gram-negative *Photobacterium damsela* subsp. *piscicida*. Photobacteriosis outbreaks can decimate entire productions, leading to massive losses and economic costs. Limited strategies can be employed on the mitigation of such outbreaks. A promising approach for the industry are functional feeds as prophylactic alternatives. Functional diets with algae derivatives can enhance the immune status and growth, helping in the prevention and outbreak mitigation of pathogen-related diseases.

This study aimed to evaluate how aqueous extracts of *Tetraselmis chui* and *Ulva* sp. combined with established commercial immunostimulants, a β -glucan-rich product and a sulphated polysaccharide product, modulate the immune response and disease resistance after a short term feeding boost followed by a pathogen insult.

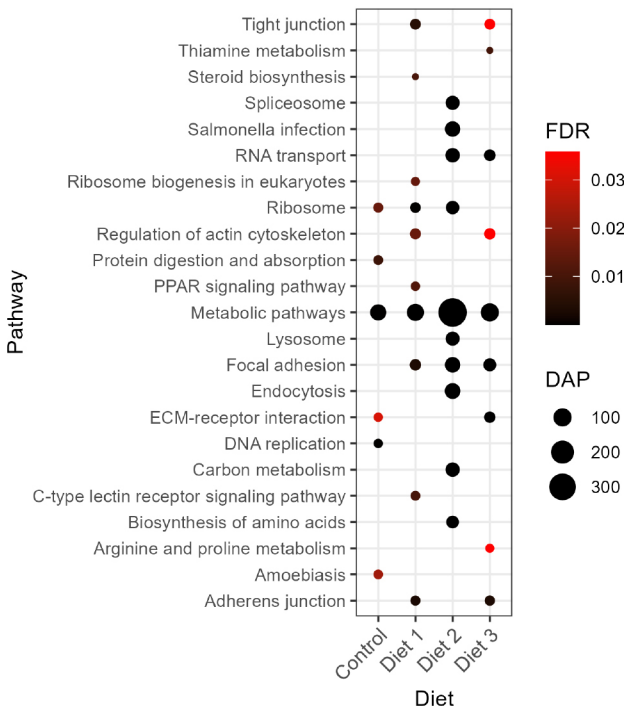


Figure 1 - Bubble chart of top 10 KEGG enrichment analysis (per diet) of differential abundant proteins (DAP) in the anterior gut of European seabass-fed experimental diet (Control, Diet 1, Diet 2 and Diet 3) 72 hours after pathogen exposure.

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Diet composition and experimental design

This study is comprised of two stages: 1) feeding trial, where fish were fed 4 experimental diets for 5 days. Fish were fed a control diet (CTRL) based on commercial formulations, the CTRL supplemented with a combination of an aqueous extract of *Tetraselmis chui* and, either, the commercially available supplement rich in β -glucans (Diet 1), or sulfated polysaccharides (Diet 2), or CTRL supplemented with a blend of *Ulva* sp. extract and the β -glucans rich product (Diet 3). Inclusion levels were within the range practiced by the industry for feed additives (ppt to ppm). Seabass were fed by hand at 3% fish body weight per day; 2) After the feeding trial, fish were orally infected with a pathogenic strain of *Photobacterium damsela* subsp. *piscicida* (strain: GRDL 19-1 (HK2)), and mortalities were monitored for 8 days. Fish were sampled at three sampling points, before infection, to assess the basal immune status after being fed dietary treatments, and at 6- and 72 hours after pathogen insult, to evaluate the immune response to infection. Liver and head-kidney samples were collected for gene expression analyses, anterior intestine for proteomics, liver for oxidative status, and plasma for humoral immune parameters.

Results and Discussion

Proteomic analysis of the anterior gut showed major modulation 72 hours after pathogen exposure, with major changes in proteins associated with gut integrity and metabolic pathways (figure 1). Gene expression of immune genes in the head-kidney showed a significant modulation with time after pathogen challenge, particularly at 72 hours after infection. Gene expression analysis of liver showed a dietary modulation in genes associated with fatty acid metabolism and energy production at the end of the feeding trial. It was observed a significant upregulation of these pathways-related genes in fish fed the experimental diets when compared to CTRL, being considerably higher in fish fed Diet 2. Genes related to pathogen recognition were also upregulated in the liver after feeding Diet 2. Taken together, data from the present study suggest that supplementing *Tetraselmis chui* extracts with sulfated polysaccharides can have a synergistic effect in enhancing fish basal immune status. However, in the presence of *Photobacterium damsela* subsp. *piscicida*, dietary modulation was not sufficient to elicit a differential response, as it was observed in the proteomic analysis and visual observation where it was noted a intestinal tissue degradation at 72 hours. These findings are consistent with disease resistance data observed after challenge, where fish survival ranged from 30.8% in Diet 3 to 17% in Diet 2 after 8 days, with no significant differences observed between treatments.

Acknowledgments

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CONSUMER PERCEPTIONS OF ALTERNATIVE AQUAFEED INGREDIENTS FOR FARMED SALMON: A CROSS-COUNTRY SURVEY IN FRANCE, THE UNITED KINGDOM AND GERMANY

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Introduction

Global demand for aquatic foods is set to grow substantially, with the 2025 EAT–Lancet Commission projecting a 46% increase in aquatic food production to meet planetary health targets by 2050 (Rockström et al., 2025). Meeting this demand sustainably requires further diversification of aquafeed formulation. Salmon farming has traditionally relied on fishmeal and fish oil for essential EPA and DHA, critical for fish health and human nutrition (Santigosa et al., 2023). Over two decades, the European industry reduced marine ingredient dependency by shifting to plant inputs, but this increased land use, carbon emissions, and eutrophication (Kok et al., 2026), while EPA and DHA levels in farmed salmon declined (Hannisdal et al., 2025). The sector must now optimise for both nutrition and environment, requiring a broader basket of ingredients. Promising alternatives exist for both omega-3 supply and protein (Glencross et al., 2023), yet adoption remains limited by cost, retailer restrictions, and perceived consumer rejection. In multi-stakeholder workshops facilitated by Earthworm Foundation involving retailers, processors, farmers, and feed producers, consumer perception consistently emerged as a major barrier to feed diversification yet empirical evidence on actual consumer attitudes remains scarce. This study addresses that gap in the three major European salmon consumer markets responsible for these value-chain restrictions: France, the UK and Germany.

Materials and Methods

Three nationally representative online panel surveys were conducted: in France (July 2024, n = 1,000), the United Kingdom (May 2025, n = 1,000) and Germany (March 2026, n = 1,050); analyses were run on the 2,569 respondents who eat salmon. Quota sampling was applied based on gender, age, socio-professional category, region of residence, and urban area size, in line with national statistics (margin of error: 3%, confidence level: 95%). Data collection was managed by BVA Xsight (France, UK) and Moweb (Germany). The questionnaire comprised five sections: sociodemographic profile, seafood consumption habits, aquaculture and aquafeed knowledge, ingredients acceptance (three for fish oil: microalgae oil, GM plant oil, farmed salmon oil; three for fishmeal: soybean meal, insect meal, poultry meal), and psychographic profile. Data analysis included descriptive statistics, chi-square tests, logistic regression per ingredient, and mixed-effects logistic regression across ingredients with a respondent random intercept, applying Benjamini-Hochberg correction for multiple testing (RStudio, $p < 0.05$).

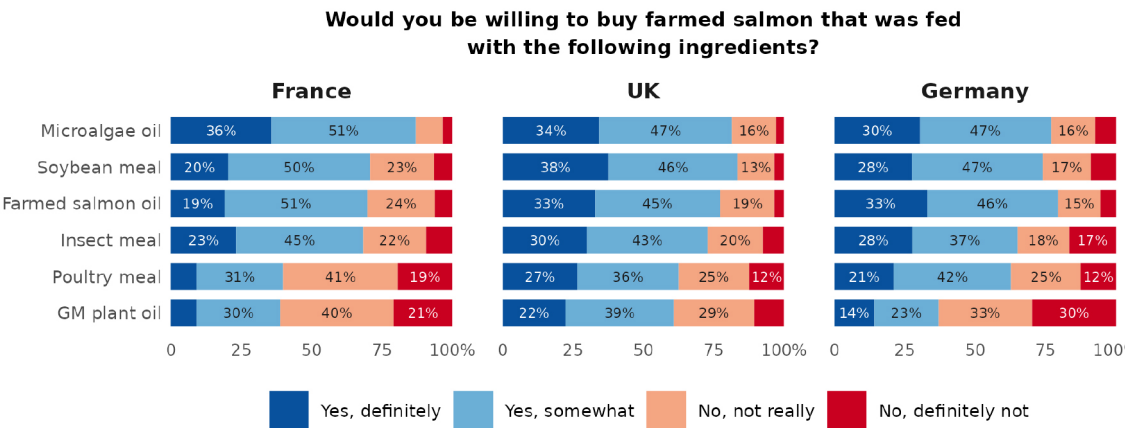


Figure 1. Willingness to buy farmed salmon fed with alternative ingredients in France, the UK and Germany.

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Results

Willingness to buy salmon fed with alternative ingredients was high overall, with notable cross-country differences (Figure 1). Four of the six ingredients were accepted by majorities in all three countries: microalgae oil (77–87%), soybean meal (71–84%), farmed salmon oil (70–79%) and insect meal (65–73%). Resistance was confined to GM plant oil and poultry meal. For GM plant oil, France (39%) and Germany (37%) were statistically indistinguishable while the UK reached 61%; for poultry meal, Germany (63%) and the UK (62%) were indistinguishable while France fell to 40%. Willingness to buy was driven principally by attitudes rather than demographics, with a positive view of farmed fish increasing it and opposition to GMOs reducing it; most effects differed in magnitude between countries. Opposition to GMOs reduced willingness to buy for all six ingredients.

Discussion

These results provide the first cross-country empirical evidence that consumer willingness to buy salmon fed with alternative aquafeed ingredients is higher than commonly assumed by industry actors. While multi-stakeholder consultations consistently identify consumer rejection as a primary barrier to feed diversification, majorities in all three countries would purchase salmon fed with most tested alternatives. Farmed salmon oil, restricted by several retailers on the assumption that consumers reject it, was among the most accepted ingredients tested and the first choice among fish oil alternatives for German and UK consumers. Where resistance does occur it is both ingredient-specific and country-specific, so a single European consumer position cannot be invoked to justify pan-European sourcing specifications. Younger consumers, who are also among the most frequent salmon buyers, show significantly higher acceptance of the two contested ingredients, suggesting that resistance may diminish as this segment matures. Acceptance was driven by attitudes rather than by knowledge, which had no independent effect once attitudes were accounted for. These findings challenge the precautionary stance constraining feed reformulation in European salmon supply chains and offer an evidence base for more sustainable aquafeed strategies.

Acknowledgment

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ARE WE MISMEASURING ENVIRONMENTAL SUSTAINABILITY IN AQUACULTURE?

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Aquaculture is widely promoted as a more environmentally sustainable solution to meet the growing global demand for animal protein when compared to terrestrial food systems. However, this narrative is often grounded in generalised assumptions and aspirational claims rather than in consistently quantified evidence. Even when sustainability is assessed, current approaches may be fundamentally incomplete. Life Cycle Assessment (LCA) is the dominant approach to environmental sustainability assessment in aquaculture research and industry. It provides quantified indicators of environmental impacts, some of which are understood and accepted by the general public, such as greenhouse gas emissions. Yet, by focusing primarily on environmental burdens per a functional unit, LCA overlooks the ecological processes and biophysical inputs that sustain production systems. In particular, it provides limited visibility into the quality of resource inputs, the distinction between renewable and non-renewable environmental support, and the broader ecological dependencies underlying aquaculture systems.

Emergy (with “m”) synthesis offers a complementary perspective to LCA by quantifying the environmental work embodied in both natural and economic inputs, expressed in solar emjoules. This approach explicitly distinguishes between renewable environmental flows, non-renewable local resources, and purchased inputs, thereby revealing system dependency and environmental loading. Emergy synthesis has demonstrated that aquaculture systems vary significantly in their reliance on ecological versus industrial support, a dimension that is not fully captured by impact-based approaches.

Aquaculture systems operate at the interface between natural ecosystems and industrial production, resulting in a wide spectrum of system configurations and resource dependencies. For instance, systems range from extensive pond systems that rely heavily on ecosystem metabolism to intensive recirculating systems dependent on external energy and feed inputs. This variability highlights that sustainability, particularly in aquaculture but certainly not limited to it, cannot be fully understood through a single analytical lens. Despite this, LCA and emergy synthesis are still rarely considered together. As a result, critical resource dependencies, system constraints, and context-specific trade-offs remain hidden, limiting our ability to effectively improve environmental sustainability in aquaculture.

This work argues that sustainability in aquaculture has been systematically misrepresented when assessed solely through impact-based approaches. It examines what current practice overlooks, explores how a more integrative perspective could be articulated, and reflects on the implications of this shift for improving sustainability assessment and decision-making in aquaculture. Rather than proposing a formal methodological framework, we position LCA and emergy as complementary perspectives that can, and should, be applied together, offering a more comprehensive understanding of both environmental impacts and ecological support (Figure 1).

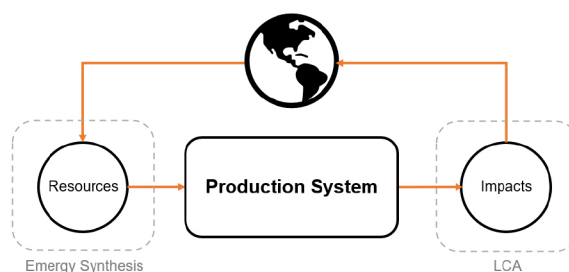


Figure 1. Integrating emergy and LCA to jointly capture ecological support and environmental impacts in aquaculture systems.

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Current practice overlooks the divergence between environmental impact intensity and system dependency, which becomes evident when impact-based metrics are interpreted alongside emergy-derived indicators. Systems characterised by low impacts per functional unit may still exhibit high environmental loading and strong dependence on non-renewable or high-transformity inputs, including compounded contributions from feed production, energy use, and water sourcing. This misalignment suggests that apparent efficiency gains can mask underlying structural dependencies, limiting the ability of current assessments to identify system vulnerabilities and long-term sustainability constraints.

A more integrative perspective can be articulated by interpreting LCA and emergy synthesis results in parallel within a consistent system boundary, allowing environmental impacts and ecological inputs to be evaluated simultaneously. This dual-perspective approach enables the identification of mismatches between impact performance and ecological support, particularly in systems where key drivers such as feed and energy act as both major contributors to emissions and dominant sources of embodied environmental work. By linking these dimensions, the integration provides a more refined basis for comparing aquaculture systems, distinguishing between improvements driven by efficiency gains and those aligned with reduced dependency on non-renewable environmental support.

The integration of these methods has important implications for sustainability assessment and decision-making in aquaculture. It enables the identification of systems that may be optimised for impact reduction but remain highly dependent on externalised environmental support, as well as systems that operate with lower ecological loading despite moderate impact profiles. This distinction is critical for guiding system design, informing sustainability certification, and supporting policy decisions that prioritise long-term resilience over short-term efficiency gains. Ultimately, advancing sustainability assessment in aquaculture requires moving beyond partial metrics and adopting approaches that explicitly capture the interaction between environmental impacts and ecological foundations.

A DECADE OF HABREPORTS: EARLY WARNING OF HARMFUL ALGAL BLOOMS AND BIOTOXINS FOR AQUACULTURE

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Introduction

Harmful algal blooms (HABs) are increases in the density of certain phytoplankton species that are harmful to humans or our use of the marine environment. In many locations, these blooms are of particular concern to the finfish and shellfish aquaculture industries. The phytoplankton genera that are detrimental to these two related sectors are, typically, distinct. Some HAB species cause “shellfish poisoning” that results from the human consumption of shellfish that have ingested toxic cells and then bio-accumulated the toxin within their flesh. Farmed fish are impacted, in general, by different HAB species through physical interference, deoxygenation or ichthyotoxicity.

The negative impacts of a human poisoning event on the shellfish industry can be far reaching. The economic consequences of HABs for the finfish industry can be very high, for example a massive fish kill in Chile in 2016 following a bloom of the dictyochophyte *Pseudochattonella* caused the mortality of 39 million salmon with a cost of \$ (USD) 800 million.

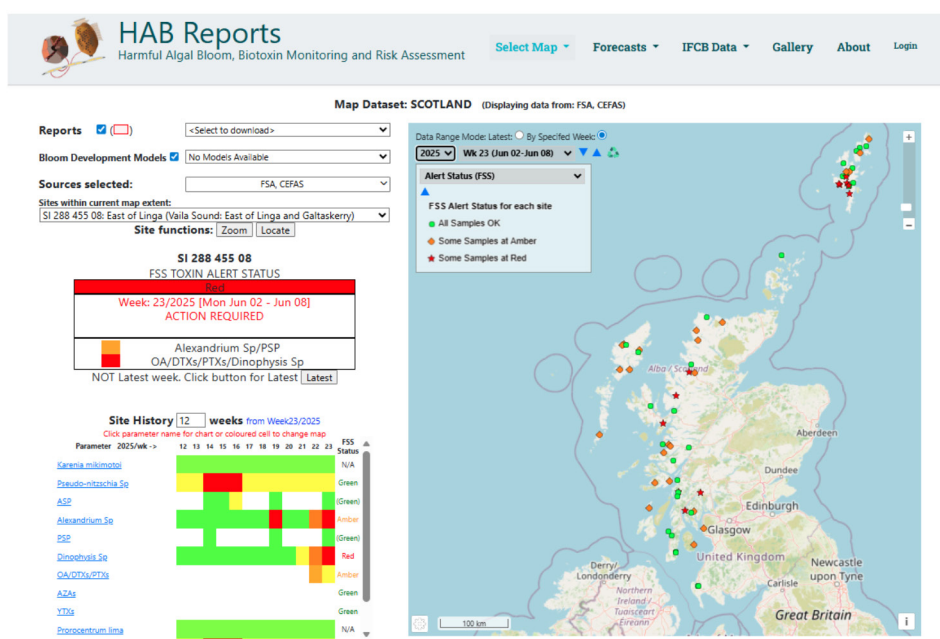
Early warning of the timing, location and magnitude of HABs and their associated biotoxins is therefore of great importance to the aquaculture industry to allow mitigation to be put in place.

Methods, results & discussion

Since 2015 SAMS has produced early warning and risk assessments of HABs and associated biotoxins in Scottish waters for the shellfish and finfish aquaculture industries. Since 2017 these have been provided on the www.HABreports.org web site (Davidson et al. 2021, Figure 1), with a parallel Malaysian (My-HABs) alerts system being developed in 2021/2.

This presentation will discuss the development and content of HABreports including its traffic light alerts, and development of a mobile phone-based App for those users who do not routinely sit at a desktop.

Figure 1: screen grab of www.HABreports.org web site



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An important component of our alert system is mathematical models. Lagrangian based unstructured grid (FVCOM) based models are used to simulate the spatial and temporal development of existing blooms to evaluate if and when aquaculture sites may be impacted. In addition, statistical ensemble models using Bayesian and other machine learning techniques are being used within HABreports to predict the onset of blooms at aquaculture prior to their occurrence (Szewczyk et al. 2025).

High resolution and taxonomically detailed monitoring of HAB taxa is crucial to early warning and risk assessment. Recent developments of our alert system have therefore incorporated automated phytoplankton imaging using two Imaging FlowCytobots sited in the aquaculture intensive Shetland Islands. AI based analysis of these image data using a neural network based on the Multi-Axis Vision Transformer allows real time reporting of HAB taxa at a temporal frequency of less than an hour (Groves et al. 2026).

The HABreports system therefore provides rapid, spatially and temporally resolved, monitoring and model based information that informs husbandry practices and business planning within the aquaculture industry protecting both human and fish health.

Acknowledgment

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PREDATORY PROTISTS RESTRUCTURE MICROBIOMES AND SHAPE EARLY-STAGE SURVIVAL OF DULSE (*P. palmata*)

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Early life stages of macroalgae depend on microbial communities that mediate their settlement, development, and survival, yet the ecological processes structuring these communities still remain poorly understood. In particular, the role of microbial predators in structuring host-associated microbiomes has rarely been tested experimentally, despite the ubiquity of predators in natural systems.

Here, we disentangle bottom-up and top-down controls on early macroalgal holobiont assembly and host survival using replicated, closed microcosms of the model Rhodophyte seaweed *Palmaria palmata*. We manipulated settlement substrate type, and trophic structure by independently adding probiotic bacterial communities ('microbial priming'), bacterivorous protists and eukaryovorous protists prior to and during the initial colonisation and settlement of *P. palmata* spores, respectively. Community dynamics were tracked over ten weeks using 16S and 18S rRNA gene sequencing alongside measurements of host settlement, growth, and survival rates.

Trophic complexity fundamentally restructured microbiome assembly and host outcomes. Notably, substrate type influenced spore settlement density; and bacterivorous protists increased bacterial diversity and homogenised community composition, consistent with predator-mediated regulation of lower trophic levels. Whereas eukaryovorous protists enhanced early host survival, likely by suppressing bacterivores and stabilising beneficial bacterial assemblages.

Together, these results demonstrate that microbial predators play a central yet underappreciated role in shaping early holobiont assembly and host survival. Rather than destabilising microbiomes, trophic interactions can promote diversity and host survival by regulating microbial dominance. These findings highlight the importance of incorporating multi-trophic interactions into microbiome ecology and suggest that early predator-mediated processes can have lasting consequences for host recruitment and resilience.

AMPHIBIOUS TECHNOLOGIES FOR SUSTAINABLE AQUACULTURE: INTEGRATING SATELLITE DATA AND IN SITU MONITORING THROUGH THE MAR (MULTIPURPOSE AMPHIBIOUS ROVER)

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Introduction

This work presents the integration of satellite data and in situ measurements for improving coastal aquaculture monitoring and management. The MAR (Multipurpose Amphibious Rover), a fully electric amphibious platform, enables high-resolution environmental surveys in complex coastal areas. By supporting validation and calibration of satellite-derived data, MAR enhances the accuracy of water quality mapping. Its real-time sensing and data processing capabilities provide a robust tool for sustainable, data-driven aquaculture.

The project

The development of European aquaculture increasingly requires advanced technological solutions capable of improving environmental sustainability, operational safety, and data-driven decision-making. In this context, the integration of satellite-based observations with high-resolution in situ measurements represents a key opportunity to enhance the monitoring and management of coastal aquaculture systems.

The MAR – Multipurpose Amphibious Rover (www.eco-mar.it) is an innovative, modular, fully electric amphibious platform designed to operate in complex coastal environments such as lagoons, estuaries, and intertidal zones. These areas are often difficult to access and insufficiently covered by traditional monitoring infrastructures, making them ideal candidates for combined remote sensing and ground-truthing approaches.



MAR equipped with a multiparametric probe and with an underwater ROV

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This contribution explores the role of MAR as a complementary tool to satellite data, enabling the validation, calibration, and optimization of remote sensing products for aquaculture applications. By performing targeted in situ surveys, MAR provides high-frequency, geo-referenced environmental measurements that can be directly compared with satellite-derived parameters, improving the accuracy and reliability of coastal water mapping.

Key application domains include:

- Integration of satellite and in situ data for enhanced mapping of coastal water quality parameters (e.g., turbidity, chlorophyll, temperature, and detection of pollutants such as microplastics);
- Real-time environmental monitoring through onboard sensors, supporting continuous assessment of ecosystem health;
- Advanced data processing and transmission capabilities, enabling near real-time analysis and decision support;
- Surveillance and inspection of aquaculture infrastructure, combined with environmental data acquisition;
- Support for the optimization of site selection and farm management through improved spatial and temporal resolution of environmental information.

By bridging the gap between satellite observation and field data acquisition, MAR contributes to the development of a more resilient, transparent, and sustainable aquaculture sector. Its application supports the objectives of the European Green Deal and the Blue Economy, fostering innovation in coastal monitoring and strengthening collaboration between research institutions, technology providers, and aquaculture stakeholders.

The presentation aims to promote integrated pilot projects in Mediterranean regions, to demonstrate the added value of combining satellite analytics with autonomous amphibious platforms for next-generation aquaculture management.

REFERENCE GENE VALIDATION FOR BYSSUS-RELATED GENE EXPRESSION ANALYSIS UNDER OCEAN ACIDIFICATION IN *Mytilus galloprovincialis*

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Background

Ocean acidification is one of the major challenges affecting shellfish aquaculture. In mussels, reduced seawater pH may alter several physiological functions, including byssus production by the foot byssal glands. Changes in byssus performance under acidified conditions have been reported, although the molecular mechanisms underlying these effects remain uninvestigated. Gene expression analyses are useful to investigate the regulation of byssus-associated proteins under future ocean acidification scenarios, although their reliability depends on the identification of stably expressed reference genes (RGs) for data normalization. Several candidate RGs have been proposed for *M. galloprovincialis* foot tissue [1]. However, their stability under ocean acidification remains to be assessed. The present study aimed to evaluate the stability of nine RGs [Actin B (*Actb*); Elongation Factor 1 α (*Ef1 α*); Cyclophilin A (*CypA*); Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*); Large Ribosomal Subunit protein eL14-like (*Rpl14*), eL32-like (*Rpl32*), eL34-like (*RPL34*); Small Ribosomal Subunit protein eS4-like (*Rps4*), and Small Ribosomal Subunit rRNA (*18S rRNA*)] in mussel exposed to seawater acidification, using hydrolysis probe-based RT-qPCR. The acidification treatment was chosen to simulate provisional surface pH for 2300 (pH 7.4), following the IPCC projections [2].

Materials and Methods

Mediterranean mussels (*M. galloprovincialis*) were purchased alive from a commercial farm (Sabaudia, Tyrrhenian Sea), randomly assigned to three tanks of a closed and controlled recirculating system, and acclimated for 10 days under control conditions (pH 8.2, 20.1 $\pm$ 0.3 °C). Mussels were fed once a day *ad libitum* with Shellfish Diet 1800® (Reed Mariculture Inc., USA). At the end of the acclimation, pH in the experimental treatment was progressively reduced by CO₂ injection into the system (0.017 pH units/h) and maintained at pH 7.4 (20.1 $\pm$ 0.3 °C). After 7 days of exposure, 5 mussels per treatment were sacrificed, the foot was excised, weighed, and immersed in RNA later solution for 24 h at 4°C. Total RNA was isolated with the GRS total RNA kit (GRISP) and quantified by the QuantiFluor® RNA 2.0 (Promega), using the Quantus Fluorometer (Promega). cDNA was synthesized through the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystem), and qPCR was performed by the Xpert Fast Probe (GRISP) on the StepOne instrument. Primer sequences were selected based on previously published results [1], while custom probes were designed using Primer 3 [3]. According to the MIQE guidelines, candidate RGs were evaluated by RT-qPCR across all experimental samples. Gene expression stability was assessed using geNorm [4], NormFinder [5], BestKeeper [6], and RefFinder [7]. To avoid bias associated with co-regulated genes, the final selection considered both the algorithms' ranking and the biological function of the candidate genes.

Results and Discussion

Amplification efficiencies of the selected genes ranged from 90% to 105%, confirming the suitability of the newly designed probes for RT-qPCR analyses. Gene stability assessment produced largely consistent results across algorithms. geNorm identified *Rpl32* (M = 0.1654) and *Rpl34* (M = 0.1701) as the most stable reference genes, followed by *Ef1 α* (M = 0.1721). Similarly, NormFinder ranked *Rpl32* (stability value = 0.169) and *Rpl34* (0.228) as the most stable genes, with *Ef1 α* (0.358) also showing high stability. BestKeeper analysis revealed the highest correlation coefficients for *Rpl32* (r = 0.995), *Ef1 α* (r = 0.985), *Rpl34* (r = 0.984), and *Rpl14* (r = 0.983), whereas *Gapdh* and *18S rRNA* exhibited the lowest stability across all methods. Integration of geNorm, NormFinder, BestKeeper, and Δ Ct rankings through RefFinder identified *Rpl34*, *Rpl32*, and *Ef1 α* as the three most stable candidate RGs. Although *Rpl34* and *Rpl32* occupied the top two positions in the comprehensive ranking, both encode ribosomal proteins and may therefore be subjected to co-regulation. Notably, *Ef1 α* was consistently ranked among the most stable genes by geNorm, NormFinder, and BestKeeper, supporting its selection as an independent normalization partner, minimizing potential bias associated with the co-regulation.

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Conclusion

The probe-based assay developed in this study showed high efficiency and reliable performance. The combined application of geNorm, NormFinder, BestKeeper, and RefFinder enabled the identification of robust RGs for expression studies in the foot tissue of *M. galloprovincialis* exposed to acidified seawater. Among the candidate genes, *Rpl34*, *Rpl32*, and *Efla* displayed the highest expression stability across all algorithms. Considering both stability and biological independence, *Efla* combined with either *Rpl34* or *Rpl32* is proposed as a robust normalization strategy for future studies. These findings provide a framework for investigations on the molecular mechanisms underlying mussel responses to ocean acidification, including those involved in byssus production.

Acknowledgment

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SEAWATER ACIDIFICATION IMPACTS MEDITERRANEAN MUSSEL (*M. galloprovincialis*) OXIDATIVE STATUS AND IMMUNE FUNCTION

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Background

Climate change affects the health of marine bivalves through a variety of environmental stressors. Among them, ocean acidification is considered one of the most pervasive long-term drivers of change to marine ecosystems. It is caused by the continuous absorption of atmospheric CO₂, which lowers seawater pH and alters carbonate chemistry. This can impair physiological functions and reduce the ability of marine organisms to cope with additional stressors [1]. As sessile filter feeders, mussels are particularly vulnerable to fluctuations in seawater chemistry, making them valuable bioindicators for assessing the biological consequences of ocean acidification.

Understanding the physiological and cellular mechanisms underlying tolerance to acidified conditions is therefore essential for the development of effective mussel farming management. This study aimed to investigate the effects of short-term and long-term exposure to reduced seawater pH on hemocyte viability, oxidative status, and innate immune responses in mussels. The experimental conditions were chosen to simulate the provisional surface pH for 2100 (pH 7.8) and the worst-case emission scenario for 2300 (pH 7.4), following the IPCC projections [2,3].

Materials and Methods

Mediterranean mussels (*Mytilus galloprovincialis*) were purchased alive from a commercial farm (Sabaudia, Tyrrhenian Sea), randomly assigned to three tanks of a closed and controlled recirculating system, and acclimated for 10 days under control conditions (pH 8.2, 20.1 ± 0.3 °C). Mussels were fed once a day *ad libitum* with Shellfish Diet 1800® (Reed Mariculture Inc., USA). At the end of the acclimation, whilst the water temperature remained unchanged, pH in the two experimental treatments was progressively reduced to target values (7.8 and 7.4) and then maintained constant by CO₂ injection. Hemolymph was sampled at short-term (10 days; T1) and long-term (35 days; T2) acidification exposure, as previously described [4]. Hemocyte viability was evaluated through the Neutral Red Uptake (NRU) assay, and Optical Density (OD) was measured with a Multiskan (ThermoFisher). Reactive Oxygen Species (ROS) production was assessed using CellROX® probe, while phagocytic activity was determined using pHrodo™-conjugated *E. coli*. In addition, phagocytosis-related oxidative burst was evaluated by subtracting the basal ROS signal from the CellROX® phagocytosis-related signal. Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Fluorescence emission was detected with the Glomax fluorometer (Promega).

An exploratory principal component analysis (PCA) was used to assess the multivariate dataset. Hemocyte immune responses were then analysed using linear mixed-effects models including pH, exposure time, and their interaction as fixed effects, and tank as a random effect. Significance of fixed effects was assessed using Type III F-tests with Satterthwaite's approximation, followed by Tukey-adjusted pairwise comparisons of estimated marginal means.

Results and Discussion

Compared to the control condition (pH 8.2), hemocyte viability was significantly reduced under the pH 7.4 treatment, regardless of exposure time, indicating that severe acidification impaired lysosomal integrity and cellular metabolic activity. Basal ROS production progressively increased with decreasing pH during short-term exposure. In contrast, no significant differences were detected after long-term exposure, which may reflect a physiological acclimation to prolonged acidification. Hemocytes did not show alteration in phagocytic capacity after short-term exposure, suggesting that compensatory mechanisms may initially preserve immune function under acidified conditions. However, phagocytic capacity was significantly reduced in both acidified treatments after long-term exposure. A similar pattern was observed for oxidative burst, which remained unchanged after short-term exposure but significantly decreased in both acidified conditions following long-term exposure, reflecting an impaired hemocyte innate immune function under prolonged seawater acidification.

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Conclusion

Overall, our results indicate that prolonged seawater acidification impairs hemocyte immune function in *M. galloprovincialis*, whereas short-term exposure elicits adaptive responses that temporarily preserve key cellular functions. These findings highlight the importance of considering exposure duration when assessing the biological impacts of ocean acidification and suggest that chronic acidification may increase mussel susceptibility to environmental stressors and pathogens. By providing new insights into the temporal dynamics of mussel immune responses under acidified conditions, this study contributes to a better understanding of the resilience of this species under future ocean acidification scenarios and supports the development of more sustainable and climate-resilient aquaculture strategies.

Acknowledgment

This study was funded by the Ministry of Agriculture, Food Sovereignty and Forests (MASAF, Italy), project OPTIFARM (Grant N. J87G624000030001).

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SHELL MECHANICAL PERFORMANCE UNDER ACIDIFICATION: EVIDENCE OF FUNCTIONAL STABILITY DESPITE ALTERED MORPHOMETRIC-RESISTANCE RELATIONSHIPS IN *Mytilus galloprovincialis*

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Background

Ocean acidification (OA) reduces seawater carbonate saturation, increasing the energetic costs of biomineralization and potentially compromising shell formation in marine calcifiers. In mussels, previous studies have reported alterations in shell mineralogy, ultrastructure, thickness, and mechanical properties under prolonged OA exposure, suggesting reduced shell protective capacity [1-3]. However, the extent to which these structural changes translate into measurable alterations of shell mechanical performance during shorter exposure periods remains poorly understood. This knowledge gap is particularly relevant for aquaculture, where shell integrity influences animal survival, handling, resistance to mechanical stress and ultimately, overall production yields. Through an integrated assessment of physiological responses to OA, the OPTIFARM project seeks to identify resilience- and vulnerability-related traits in Mediterranean mussels. In this study, we focused on shell mechanical resistance, investigating whether 35 days of exposure to reduced seawater pH alters shell compression resistance.

Materials and Methods

A total of 238 adult mussels were reared in a controlled closed system and exposed for 35 days (after a 10-day acclimation period) to three seawater pH treatments (8.2, 7.8, and 7.4 [4,5]), with three replicate tanks. Mussels were sampled before exposure (T0), after 7 days (T1, short-term exposure) and after 35 days (T2, long-term exposure). Compression resistance was measured on each valve using an Instron 5543 operated through Bluehill software. Shell area (left valve only) was quantified from digital images, and shell thickness was measured at three anatomical shell regions (posterior margin, median, and the umbo) using a digital calliper. Statistical analyses were performed in R. Shell compression resistance was analysed using linear mixed-effects models including pH, exposure time, shell side, and shell thickness as fixed effects, and mussel identity as a random effect. The relationship between shell morphology and compression resistance was further investigated using linear models testing the interaction between shell area and pH. Model assumptions and the robustness of significant relationships were evaluated through residual diagnostics and sensitivity analyses.

Results

Under the conditions tested, shell compression resistance was not significantly affected by seawater pH ($p = 0.442$), exposure time ($p = 0.058$), or their interaction, indicating that shell mechanical performance was maintained throughout the experiment. Shell thickness at the medial ($p = 0.009$) and umbo ($p = 0.010$) regions was positively associated with compression resistance, whereas posterior shell thickness showed no significant effect. In contrast, the relationship between shell size and mechanical performance differed among pH treatments (shell area $\times$ pH interaction, $p = 0.023$): under control (pH 8.2) and moderately acidified conditions (pH 7.8), compression resistance increased with shell area, whereas this relationship was no longer observed under pH 7.4. Sensitivity analyses confirmed that this pattern was robust to influential observations. These findings suggest that severe acidification does not immediately impair shell mechanical performance but may decouple shell growth from mechanical reinforcement.

Discussion and conclusions

Previous studies have shown that prolonged ocean acidification alters shell biomineralization, crystal organization, and shell composition, leading to reduced shell mechanical properties in *M. coruscus* after 40 days of exposure [3], and in *M. edulis* after 6-9 months of exposure [1-2]. In contrast, our findings indicate that shell compression resistance was maintained over 35 days of exposure in *M. galloprovincialis*, despite significant changes in the relationship between shell morphometric traits and mechanical performance. This suggests that functional shell performance may be preserved during the early stages of ocean acidification exposure. However, given that ocean acidification is a decadal-scale process according to IPCC (Intergovernmental Panel on Climate Change) projections, longer experiments integrating biomechanical, mineralogical and microstructural analyses are needed to determine whether the observed altered morphology-performance relationships precede measurable declines in shell strength. Together with complementary investigations on byssus performance, immune competence and byssus-related gene expression, this study contributes to a broader understanding of the integrated physiological responses of *M. galloprovincialis* to ocean acidification.

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EFFECTS OF DIFFERENT DIETARY OIL SOURCES COMBINED WITH A HIGH HYDROPHILIC NUTRITIONAL EMULSIFIER IN TILAPIA DIETS

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Introduction

The increasing volatility in availability and pricing of dietary lipid sources presents a challenge for aquafeed formulation. Limited availability of traditional oils, combined with increasing competition from other industries such as biofuel production, requires nutritionists to frequently adjust dietary oil compositions. However, changes in dietary lipid sources may affect nutrient digestibility, growth performance, and fish health.

One potential strategy to increase formulation flexibility is the use of nutritional emulsifiers with a high hydrophilic-lipophilic balance (HLB), which may support lipid emulsification and micelle formation under varying dietary fat compositions, leading to better digestion and absorption of the fats. The present study evaluated the effects of different dietary oil combinations, with or without supplementation of a high HLB nutritional emulsifier, on the performance of red tilapia (*Oreochromis mossambicus* x *Oreochromis niloticus*).

Material and methods

The trial was conducted at Nam Aig Farm, part of Nam Sai Farms, Thailand, under commercial grow-out pond conditions. Each dietary treatment was tested in quadruplicate using ponds measuring 4 × 3 m with a water depth of 1.2 m and stocked with 300 fish per pond. The feeding trial lasted 24 weeks, during which fish grew from an initial average body weight of 43 g to a final harvest weight of approximately 700 g.

Fourteen experimental diets were formulated to be isonitrogenous and isolipidic, differing only in dietary oil composition and emulsifier inclusion level. A total added oil inclusion of 3% was maintained in all treatments and consisted of either 3% fish oil, 1% fish oil combined with 2% soybean oil, 1% fish oil combined with 2% palm oil, or 3% soybean oil. Diets were supplemented with 0, 250, 350, or 500 ppm of a high HLB nutritional emulsifier (Volamel Extra Aqua, Nukamel BV, The Netherlands), while the treatment with complete fish oil replacement by soybean oil was evaluated at 0 and 500 ppm only.

Water quality parameters, including dissolved oxygen and water temperature, were monitored throughout the study but not artificially controlled.

Evaluated parameters included growth performance, feed efficiency, survival, and water quality. Data were analyzed using ANOVA. Significance was declared at $P \leq 0.05$ and a trend to significance at $0.05 < P \leq 0.1$.

Results

Changes in dietary oil composition resulted mainly in numerical differences in growth performance and feed efficiency, while survival remained largely unaffected. In general, diets containing combinations of fish oil and plant oils showed improved tank biomass and feed conversion ratio (FCR) compared to diets containing only fish oil or soybean oil.

Supplementation with the high HLB nutritional emulsifier influenced growth performance, feed efficiency, and survival depending on dietary oil composition. The response to emulsifier inclusion did not follow a consistent linear dose-response pattern across all oil types, suggesting that optimal inclusion levels may depend on dietary lipid source.

In fish oil-based diets, emulsifier supplementation significantly improved FCR, while mixed oil diets showed improvements in tank biomass, mean body weight, and FCR at higher inclusion levels although optimal inclusion levels differed between oil sources. In diets containing palm oil, supplementation with 500 ppm emulsifier significantly improved tank biomass and survival. In the complete soybean oil replacement diet, emulsifier supplementation significantly reduced FCR and numerically improved growth performance.

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Conclusion

The present study demonstrated that changes in dietary oil composition only numerically affected the performance of red tilapia, indicating a high level of tolerance towards varying dietary lipid sources. Supplementation with a high HLB nutritional emulsifier improved feed efficiency and performance parameters across multiple dietary oil combinations.

The response to emulsifier supplementation did not follow a consistent linear dose-response pattern across different oil types, suggesting that optimal inclusion levels depend on dietary oil source and fatty acid composition. Partial replacement of fish oil with soybean oil appeared to respond favorably to intermediate emulsifier inclusion levels (350 ppm), whereas diets containing palm oil or complete replacement of fish oil by soybean oil showed more consistent responses at higher inclusion levels (500 ppm). Further research is needed to better understand the interaction between dietary lipid composition, emulsifier supplementation, and tilapia performance under commercial production conditions, in a pond system.

UNIQUE SCIENTIFIC AND TECHNICAL INFRASTRUCTURE FOR ATLANTIC BLUEFIN TUNA (*Thunnus thynnus*) AQUACULTURE

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Introduction

The Atlantic bluefin tuna (*Thunnus thynnus*, ABFT) is an iconic species that has sustained human populations in the Mediterranean for millennia. Overfishing severely depleted wild stocks, but recovery measures adopted by ICCAT in 2006 have helped restore populations. However, increasing market demand can only be sustainably met through full-cycle aquaculture. Therefore, developing reliable breeding and farming techniques that complete the species' entire life cycle in captivity is essential for the future of bluefin tuna production.

The singular scientific and technical infrastructures (ICTS) are large installations, resources, facilities and services, unique in its kind, that are dedicated to cutting edge and high-quality research and technological development, as well as to promote exchange, transmission and preservation of knowledge, technology transfer and innovation. The Infrastructure for Atlantic bluefin tuna aquaculture (ICAR), belonging to the Spanish Institute of Oceanography (IEO-CSIC), is a unique scientific and technical Infrastructure (ICTS) devoted to study the full aquaculture of ABFT (www.icar.ieo.es). Located in the Murcia Region (SE of Spain), it is constituted by the Mazarrón Marine Aquaculture Plant and the Installation for the Control of the Reproduction of ABFT (ICRA).

Objectives

The objectives of the infrastructure are to provide the scientific community at national and international level, an open access, state-of-the-art and unique facilities devoted to competitive research and technology transfer for ABFT reproduction in captivity and juvenile production, in order to contribute to the sustainable production through integrated aquaculture techniques independently of fisheries, and to increase knowledge on its biology for the application in better fisheries management, thus contributing to its sustainability.



Figure 1. From left to right and from top to bottom. ICRA, ICRA RAS system, larval rearing tanks, BFT eggs and larvae hatching, pre-ongrowing tanks, ABFT swimming in the ICRA.

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Facilities

The ICTS-ICAR has the following units:

Unit A) ABFT breeding control facility (ICRA): Four tanks with a total volume of 7,000 m³. Two tanks, 22 and 20 m in diameter and 10 m deep, intended for broodstock, with the capacity to house approximately 30 ABFT weighing around 200 kg each, and another two, 14 and 8 m in diameter, for juveniles. The facility is capable of controlling the photoperiod and temperature.

Unit B) Egg incubation unit: 12 tanks with a capacity of 500 l and 50 tanks with a capacity of 100 l, with light and temperature control.

Unit C) Larval rearing unit: 7 tanks of 5,000 l, 18 tanks of 1 m³ and two experimental modules with 12 and 27 circular tanks of 170 l each. Mesocosm unit for larval rearing, comprising two 45 m³ tanks and a facility for the cultivation of phytoplankton, rotifers, *Artemia* and copepods (*Acartia*), sized to meet the requirements of larval rearing. All tanks are equipped with water treatment and recirculation (RAS) systems.

Unit D) Weaning and pre-ongrowing facilities: One 100 m³ cylindrical tank, four 50 m³ tanks and four 20 m³ tanks, equipped with recirculation and water treatment systems, as well as light and temperature control systems.

The four units are grouped in two nearby but separated buildings. The first (ICRA) contains the facilities for controlling the reproduction of ABFT. The other building (PLANT) contains the facilities for incubation, larval rearing and weaning-preongrowing (Figure 1).

Examples of access

ABT Feeding: Effect of different foods on biochemical composition of ABFT juveniles.

ENROTUNA: Improving the enrichment of rotifers used to feed ABTF larvae (Roo *et al.*, 2024).

BLUE-HEART: Using cardiac physiology to investigate spontaneous activity patterns, routine metabolic rate, and tolerance of warming in farmed ABFT juveniles (McKenzie *et al.*, 2026).

SAVETHUN/COMATHUNA: Acoustic and computer vision for estimation and non-invasive monitoring of ABFT biomass and its interrelation with the soundscape (Espinosa *et al.*, 2025).

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EFFECTS OF POVIDONE-IODINE PROPHYLAXIS ON METABOLIC AND OXIDATIVE STRESS RESPONSES DURING INDUCED ASEXUAL REPRODUCTION OF THE SEA ANEMONE *Anemonia viridis*

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Introduction

Induced asexual reproduction offers a promising approach for the captive propagation of sea anemones, with potential applications in aquaculture, conservation, and marine restoration (Fraser et al., 2021). Longitudinal bisection can rapidly generate new individuals (Watson and Younger, 2022), but tissue injury may increase susceptibility to infection and trigger physiological stress, as wound healing and immune defense compete for limited metabolic and cellular resources (La Corte et al., 2025). Iodine-based antiseptics, such as Lugol's solution, have been used following coral fragmentation, and recent evidence indicates that their effects on fragment survival, healing, and physiology may depend on concentration and exposure duration (Lam et al., 2025). However, the physiological consequences of povidone-iodine treatment following tissue injury remain poorly understood in sea anemones. Therefore, evaluating metabolic and oxidative stress biomarkers, such as glucose, lactate, superoxide dismutase (SOD), and glutathione peroxidase (Gpx) activities (La Corte et al., 2024; Vitale et al., 2020), may provide insight into the physiological effects of povidone-iodine treatment during induced asexual reproduction in *Anemonia viridis*.

Materials and Methods

The animals used in the study were obtained from the stock maintained at IFAPA Centro “Agua del Pino” (Cartaya, Huelva, Spain). Fifty-four *A. viridis* individuals (20.96 ± 1.55 g, mean $\pm$ SEM) were randomly assigned to two groups ($n = 27$). All individuals were longitudinally bisected. Fragments from one group were treated with povidone-iodine (0.1%, 2 min), followed by rinsing with filtered seawater for 60 s, whereas fragments from the other group received no povidone-iodine treatment (control). The fragments were maintained in 12 fiberglass trays (20 L; 6 per treatment; 9 fragments per tray) under the established experimental conditions. Within each tray, three fragments were assigned to each sampling time (6 h, 24 h, and 7 days). At each sampling time, lactate and glucose levels and SOD and GPx activities were determined. Survival was monitored in the fragments assigned to the 7-day sampling point.

Results

Mortality at 7 days was 33.3% in the povidone-iodine treatment and 44.4% in the untreated control, with no significant differences between treatment and mortality (Fisher's exact test, $p = 0.733$).

Glucose showed significant effects of time ($p = 0.002$) and treatment $\times$ time interaction ($p = 0.027$). Glucose levels were significantly higher in controls than in povidone-treated fragments at 24 h ($p = 0.019$), whereas no differences were detected at 6 h or 7 days. Lactate was significantly affected by time ($p = 0.007$), but neither the treatment effect ($p = 0.318$) nor the treatment $\times$ time interaction ($p = 0.085$) was significant. At 24 h, lactate was higher in controls than in povidone-treated fragments ($p = 0.044$). SOD activity was not significantly affected by treatment, time, or their interaction ($p > 0.05$). GPx activity showed significant effects of time ($p = 0.016$) and treatment $\times$ time interaction ($p = 0.017$). At 7 days, GPx activity was significantly higher in controls than in povidone-treated fragments (0.493 vs. 0.288; $p = 0.003$).

Discussion

The higher glucose and lactate levels observed in control fragments at 24 h are consistent with an increased metabolic demand during the early response to tissue injury. The lower levels observed in povidone-treated fragments may indicate a reduced metabolic response during this initial recovery phase. At 7 days, GPx activity was significantly lower in povidone-treated fragments, while SOD activity remained unchanged. This difference may reflect a reduced antioxidant demand during recovery. These results suggest that povidone may modulate the physiological response during recovery without increasing mortality, supporting its potential as a prophylactic treatment for *A. viridis* fragmentation. Further studies evaluating different concentrations and exposure times are needed to identify an optimal prophylactic protocol for *A. viridis* post-cutting recovery.

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EVALUATING THE RESISTANCE OF *Ruditapes philippinarum* TO OCEAN ACIDIFICATION IN THE ADRIATIC SEA

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Introduction

Rising carbon dioxide emissions pose a critical environmental challenge leading to ocean acidification. This process can alter the physiological status of calcifying species such as bivalve mollusks, like *Ruditapes philippinarum*. The present study investigated how different pH scenarios affect the biometric parameters and behavioral functions of Manila clam, including growth, filtration, and burrowing capacity. The aim was to provide data to guide conservation strategies under ongoing ocean acidification.

Materials and Methods

A batch of 420 juvenile Manila clams, averaging 0.74 ± 0.03 g and 15 ± 1.05 mm in length were collected from the hatchery Naturesdulis located in the Goro lagoon (FE, Italy), an area with significant variability in chemical-physical parameters, especially pH.

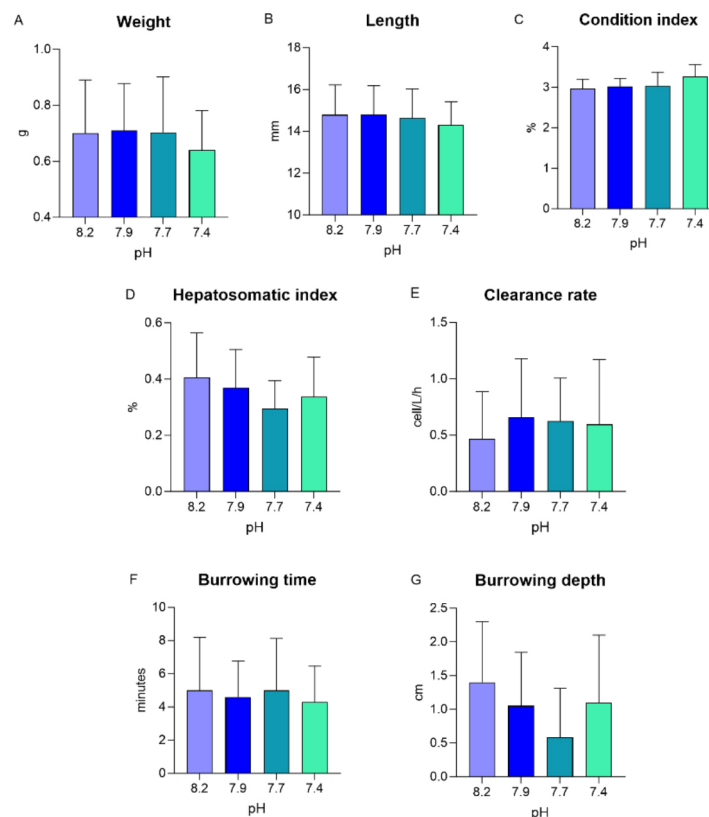


Figure 1: Total weight (A), length (B), Condition index (C), Hepatosomatic index (D), Clearance rate (E), Burrowed clams (F), Burrowing time (G), Burrowing depth (H). Data are presented as mean $\pm$ standard deviation.

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At the University of Bologna's aquaculture laboratory, clams were exposed to four pH conditions for 90 days: one control group maintained at pH 8.2 and the others exposed to pH levels of 7.9, 7.7, and 7.4. Animals were reared in four independent systems of aquaria, were fed a mixture of live microalgae. pH levels were controlled automatically by a CO₂ injection system. The temperature was maintained at 20±1°C, and salinity at 27±1‰ throughout the experiment. Biometric indices were measured at the end of the experiment, along with the condition index and hepatosomatic index, clearance rate, and burrowing behavior was observed under different pH conditions.

Results and Discussion

Statistical analysis was performed using one-way ANOVA, and Tukey's post-hoc test was applied for significant results ($p < 0.05$). The results from total weight and length at the end of the experiment did not exhibit statistical variation, even with exposure to lower pH levels. Similarly, the condition index and hepatosomatic index remained constant across all treatments, indicating no significant loss in tissue mass or energy reserves. The clearance rate test revealed no significant changes in filtration activity, suggesting that the clams maintained stable food acquisition under conditions that were not severely stressful. Behavioral responses, such as burrowing time and depth, were also unaffected by varying pH levels (Figure 1). The absence of significant changes suggests that clams were resilient to the experimental acidification conditions. This resilience may be due to the clams' origin in the dynamic Goro Lagoon, where natural pH fluctuations (ranging from a minimum of 7.06 to a maximum of 8.9 pH during the month of May) could have fostered adaptation to acidic conditions. This study demonstrates that Manila clam possesses remarkable resistance to the ocean acidification scenarios predicted for the near future. An extraordinary plasticity likely links to the origin environmental. In conclusion, although ocean acidification represents a global threat, Manila clam could tolerate significant pH fluctuations, ensuring greater stability for future shellfish farming activities.

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PROTECTING PRE-FATTENING MANILA CLAM FROM INVASIVE BLUE CRAB PREDATION: TESTING BIO-BASED ALTERNATIVES TO CONVENTIONAL COVERING NETS

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Introduction

The invasive blue crab (*Callinectes sapidus*), now widespread along Italian coasts, has become a major predator of Manila clam (*Ruditapes philippinarum*), causing severe losses to clam farming. While polyethylene covering nets are currently used to protect clam plots, these conventional plastic materials raise environmental concerns over persistence and circularity. This study evaluates bio-based alternative covering materials, assessing their resistance to crab pinching force and their compatibility with clam survival.

Materials and Methods

A total of 12,000 Manila clam individuals (T2-3 size, 0.2 cm) were used at the *NaturEdulis* controlled hatchery (Goro, Italy). Clams were distributed into rectangular tanks (56×36×32 cm external dimensions) at a density of 1,000 clams per tank over a layer of sand, in order to simulate offshore seeding conditions. Test nets (0.4 cm mesh) were positioned in each tank. The experimental design comprised four treatments, each in triplicate: *Control* (uncovered), *Polyethylene* (conventional PE net), *Biopolymer A* (Ingeo™ Biopolymer 2003D, a rigid transparent PLA derived from corn-based polylactic acid), and *Biopolymer B* (Bio-Flex® N 25370, a flexible white PLA combined with biodegradable plasticizers). The trial involved assessing predation and the mechanical resistance of the covering materials in the presence of the invasive blue crab. Medium-small male crabs (35–115 g wet weight) were used. Crabs were acclimated for one week with ad libitum feeding, followed by 24 h of fasting to stimulate predatory activity before being introduced into the tanks, three crabs per tank. Predation was monitored for 48 hours after crab introduction through continuous underwater video recording. At the end of the test, predated clams (or residual biomass weighing) were registered and visual inspection of the covering materials for perforations or lacerations caused by claw action was conducted as an indicator of mechanical resistance.

Results and Discussion

Preliminary results revealed differences among covering materials in their ability to withstand mechanical stress from crab predation. The alternative materials also showed comparable effects on water flow and clam survival to the conventional polyethylene. Video monitoring and post-trial inspection of the nets allowed direct comparison of laceration patterns and predation rates across treatments, offering initial insight into whether bio-based materials can provide protection comparable to conventional plastic covers.

Conclusion

This study will provide evidence on whether bio-based, circular-economy covering materials can serve as viable alternatives to conventional polyethylene nets in protecting pre-fattening Manila clam from blue crab predation, supporting the shellfish farming sector in adopting more sustainable practices while maintaining effective predator control.

Funding

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FAECES ON THE RISE: HOW “FLOATING FAECES” IMPROVE SOLID AND NUTRIENT REMOVAL FOR ATLANTIC SALMON PRE-SMOLT PRODUCTION IN RAS

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Introduction

Recirculating aquaculture systems (RAS) have been increasingly recognised for their properties allowing a tightly controlled environmental discharge and improving resource-use efficiency, positioning them as a highly competitive alternative to traditional systems (Ahmed and Turchini, 2021; Timmons *et al.*, 2018). However, the complex water circuit in RAS generates significant turbulence and shear forces that promote and accelerate the fragmentation of organic particulate matter. This progressive breakdown of particles reduces the effectiveness of the conventional solid removal methods, consequently increasing the possibility of solids remaining in suspension. The accumulation of these suspended solids (SS) can therefore compromise water quality and animal welfare, interfere with system performance and increase the risk of operational failure (McMillan *et al.*, 2002; Schumann and Brinker, 2020). Consequently, effective SS management remains a critical key challenge in the design and operation of RAS, requiring the continuous optimisation of the already costly treatment and filtration strategies. To address the gap of effective, energy-efficient solutions, novel strategies are needed to secure an early-stage mitigation for the accumulation of SS in RAS, while being compatible with already existing operational systems. This study was based on the “floating faeces” approach (Unger and Brinker, 2013) and investigates the effects of cork (*Quercus suber* L.) as a functional feed ingredient to reduce faecal density and facilitate a direct solid removal from a full-RAS operation husbanding the freshwater phase of Atlantic salmon (*Salmo salar* L.).

Materials and Methods

A 21-week experiment evaluated the inclusion of 3% cork granules ($\varnothing$: 0.7–1 mm) in a commercial RAS diet during the freshwater phase of Atlantic salmon. Two replicate semi-technical RAS were used. Pre-smolts (74.8 ± 11.4 g) were stocked at density of ≈ 20 kg m⁻³ and fed either a control diet or the same basal diet supplemented with 3% indigestible cork granules (+Cork). After 12 weeks, diets were switched between systems to control for system effects. Fish were hand-fed to apparent satiation three times daily and feed intake was recorded. Solids in the control were managed via bottom drains and continuous surface skimming, while the +Cork tanks were equipped with an overflow pipe for buoyant faeces removal. Faecal removal efficiency was quantified weekly over 24 hours using specialised equipment. Total suspended solids (TSS) were measured weekly before morning drainage from three locations: before the drum filter (BDF), after the drum filter (ADF), and in the backwash water (BWW). Faeces were sampled for digestibility and faecal density analyses. Water samples were taken weekly from three system points for total phosphorus (TP), total bound nitrogen (TN_b) and routine water quality analyses.

Results

The +Cork treatment produced significant amounts of floating faecal material, which was rapidly aspirated by surface pipes and directed to the drum filter. No adverse effects on feeding behaviour were observed. Growth performance and feed utilisation were consistent with commercial standards in both groups, and cork inclusion did not adversely affect growth. Feed intake (TFI) tended to be higher in Control, while the +Cork treatment significantly enhanced SGR, TGC, and PER ($p < 0.05$). In line with this improved performance, FCR was nominally lower in the +Cork group, although this difference did not reach statistical significance. Reflecting the production of floating casts, faecal density was significantly lower than that of water in +Cork ($p < 0.05$). The +Cork treatment markedly improved solids and nutrient removal: overflow removed 60.27% vs. 4.27% in Control group; drum filter removal efficiency reached 86.5% in +Cork vs. 42.4% in Control ($p < 0.001$). Pre-mechanical treatment TSS was more than 3-fold higher in +Cork ($p < 0.05$), but post-filtration TSS was significantly lower ($p = 0.034$). TP and TN_b were significantly higher before filtration in +Cork ($p < 0.05$) yet lower or similar after filtration, with greater particulate nutrient removal and reduced leaching potential ($p < 0.05$).

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Discussion

This study demonstrates, for the first time in Atlantic salmon, that incorporating 3% cork granules into a commercial RAS diet reduces faecal density below that of water ($<1.00 \text{ g cm}^{-3}$). This physical shift in faeces enables rapid surface removal via overflow pipes without requiring additional energy costs, and without compromising growth, feed utilisation, or welfare—despite a slight reduction in feed digestibility induced by the cork ingredient. By facilitating immediate surface to drum filter transport, this strategy minimised faecal exposure to tank conditions, *i.e.*, flow rates, turbulence and shear forces, thereby minimising disintegration, fragmentation and nutrient leaching. Consequently, solid and nutrient management is remarkably improved; rather than accumulating within the system, TSS and critical nutrients are partitioned into a concentrated waste stream. This is reflected in significantly enhanced water quality (reduced TSS, TAN, and CO_2). This approach can be easily applied to existing RAS and other aquaculture configurations (*e.g.* net pens). Overall, incorporating cork into commercial salmon diets represents a practical and sustainable strategy for superior waste capture and nutrient recovery, supporting cleaner production and the principles of circular economy in aquaculture.

Acknowledgement

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TOWARDS CIRCULAR SHRIMP AQUACULTURE: SLUDGE RECOVERY INTO MICROBIAL PROTEIN FOR IMPROVED PERFORMANCE AND IMMUNE RESPONSES IN *Litopenaeus vannamei*

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The sustainability of shrimp aquaculture depends on reducing waste streams while identifying alternative functional feed ingredients that support animal health and performance. Within the C-SHRIMP project, a circular production concept was developed in which sludge generated from a recirculating aquaculture system (RAS) for *Litopenaeus vannamei* was recovered and upgraded into microbial protein (MP). Waste solids collected from the RAS were concentrated through improved sludge management and subsequently subjected to controlled fermentation, where microbial biomass converted the organic fraction into a protein-rich product. This approach transforms residual aquaculture waste into a valuable feed ingredient with both nutritional and potential immunostimulatory properties. Two MP products with differing protein quality and composition were obtained and subsequently evaluated in feeding trials with whiteleg shrimp *Litopenaeus vannamei*.

Dietary trials assessed different inclusion levels of both MP products in shrimp feed (between 0.2% and 10%) and their effects on growth performance, immune responses, and disease resilience. Growth-related parameters included survival, feed conversion ratio (FCR), weight gain, and overall production performance. Only minor differences in weight gain were observed at higher inclusion levels, while survival remained unaffected across treatments, indicating good dietary tolerance. Effects of both MP products were observed on multiple performance indicators, demonstrating their biological activity in practical feeding conditions.

To evaluate immunomodulatory potential, several innate immune parameters were measured after 7 and 14 days of feeding, including total haemocyte count (THC), differential haemocyte count (DHC), superoxide dismutase (SOD), and phenoloxidase activity (POA). Both MP products influenced these immune markers, with responses depending on sampling timepoint, inclusion level, product type, and feeding duration. Changes in haemocyte profiles and enzyme activity suggest that dietary MP can modulate shrimp innate immunity and may enhance physiological readiness against stress or infection.

Following 28 days of pre-feeding with MP-containing diets, shrimp were subjected to challenge tests with two major pathogens: White Spot Syndrome Virus (WSSV) and *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease (AHPND). These trials were conducted to assess whether the observed immune effects translated into improved disease resistance.

The results demonstrate that microbial protein derived from shrimp RAS waste can be successfully incorporated into shrimp diets without compromising growth performance, while providing measurable effects on immune status and potential health resilience. This highlights the dual value of MP as both a sustainable protein source and a functional feed ingredient, supporting circular and competitive shrimp aquaculture systems.

Acknowledgement

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OPERCULAR HEALING POTENTIAL: USING GEOMETRIC MORPHOMETRY TO QUANTIFY DEVELOPMENT OF RAINBOW TROUT (*Oncorhynchus mykiss*) OPERCULA OVER TIME

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Introduction

In salmonid production, opercula shortening and deformities are a relative common (average prevalence of 7-10%), especially in the early start feeding period (Østevik et al., 2025). Potential risk factors as indicated by the industry are linked to operational and production routines such as feed allocation, tank design, water environment and current, stocking density and water temperatures. However, there is a notable lack of controlled studies specifically addressing the implications of opercular shortening and deformities on fish health and welfare.

According to Østevik et al. (2025), concerns associated with opercular abnormalities include reduced growth performance, increased susceptibility to gill-related health challenges, and diminished capacity to cope with stressors such as vaccination, handling, and environmental fluctuations. These issues may result in direct economic losses due to impaired growth and increased mortality, as well as indirect costs linked to additional management measures, including enhanced monitoring and sorting of affected fish.

To date, limited research has gone into uncovering potential biological mechanisms involved, more detailed description of shape variations observed and opercula shortening's effect on the physiological functioning of the fish. Here we present a detailed scoring system for opercular length, relating opercular deformities to changes in the gill tissue as well as documenting the ability of opercula to improve as the fish grows. These are pivotal tools to an increased understanding of the mechanisms behind opercula shortening and the consequences for the physiology of the fish.

Research aims

This study aims, by means of applying qualitative and quantitative analysis of opercula shortening, to provide insight into the improvement potential of opercula over time. In addition, the shape of gills is analyzed in relation to the opercula shortening to evaluate how opercula deformities affect the respiratory area.

Methods

At the start of the trial, fish on average 12 grams were pit tagged with 8 mm Smartrac Glass TAGs. All individuals were photographed at start and when leaving the experiment at either approximately 30 grams or 140 grams. The total length of the trial was 7 weeks. Length and weight were recorded at the same time points and minimally 2 observers independently scored the opercula shortening based on a qualitative scoring scheme. Additionally, a selection of 48 individuals, with varying severity of opercula shortening, were sacrificed for tissue sampling (operculum, second gill arch or the entire gill basket and heart) at these two time points. The tissue samples were fixed in 10% buffered formalin. Formalin fixed gill arches were photographed.

The trial included 70 individuals with initially no visual opercula shortening and 163 individuals with opercula that had varying degrees of shortening and / or other types of deformities in opercular bone structure. The opercula shortening was evaluated based on a qualitative scoring scheme. In this scheme the operculum is divided into three different zones based on the anatomical structure of the most distal opercular bones. The severity of shortening is classified from 0 (no shortening) to 4 (more than half of the gill filaments visible) in each of the three regions. Both sides of the fish were evaluated.

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The shapes of opercula and gills are currently being analyzed following the principles of geometric morphometrics using a combination of fixed anatomical landmarks and semilandmarks indicating outline curves (Webster & Sheets, 2010; Zelditch et al., 2026). A variety of anatomical landmarks on fish shape are established to which opercula landmarks can be referenced in order to visualize the extent of shortening. Analyses will focus on evaluating how different areas of the operculum are affected. Additionally, the results of the opercula shape analyses will be overlain with those of the gill arches to investigate the effect of opercula shortening on the respiratory surface of the gills.

Preliminary results and expected outcomes

Based on the results from the live scoring of the opercula shortening at the start and end of the trial, the potential for substantial improvement of the opercula has been confirmed. The group of fish selected as those with some degree of shortening of the opercula at the start (163 individuals) had on average score 3.7 (moderate to severe shortening) at both the start and 30 grams. Contrastingly, a clear improvement was seen at 140 grams with an average score of 0.6 (very slight shortening). The group of fish selected as those with no shortening at the start of the trial (70 individuals) had an average score of 0.1 (no to very slight shortening) at the start, on average 0.9 (very slight to slight shortening) at 30 grams, and 0.3 (no to very slight shortening) on average at 140 grams. When all individuals in the trial were seen as a whole the average score was 2.6 and 2.8 at the beginning and at 30 grams respectively (slight to moderate shortening) and improved over time to an average score of 0.5 (no to very slight shortening).

More detailed insights into whether each area of the operculum has similar improvement potential will be further explored by means of the morphometrics analysis currently in progress.

Based on observations made over the course of this study, and from case-by-case evaluation of fish groups at the research station, opercula shortening seems to affect the shape of the gill filaments. A quantitative assessment combining the shape analyses from both the opercula and the (second) gill arch(es) will be used to provide further insight into the extent of the effect.

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OPTIMIZING MICROBIAL MANAGEMENT IN LIVE FOOD: BIOENCAPSULATION OF FUNCTIONAL MICROORGANISMS IN ROTIFERS AND *Artemia*

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Introduction

Robust hatchery performance increasingly relies on advanced microbial management strategies. Rather than merely limiting the proliferation of harmful microorganisms, modern approaches aim to actively shape beneficial microbial ecosystems that can support animal health, improve rearing conditions and strengthen overall biosecurity. Within this framework, the targeted use of beneficial bacteria has become a key tool, contributing to a paradigm shift from corrective disease treatments towards proactive, efficiency-oriented microbial management. Live food organisms such as rotifers and *Artemia* play a critical role in early larval rearing, but they can also act as vectors for opportunistic and potentially pathogenic bacteria. Optimizing microbial control during live food production is therefore essential to reduce microbial risks at the larval stage. In this study, different strategies for the application of Sanolife MIC-F were compared in order to modulate the bacterial flora of ready-to-feed rotifers and *Artemia*, with the objective of improving bio-encapsulation efficiency of functional microorganisms and overall microbial stability of live food.

Materials and methods

Application in rotifers

Rotifers were cultured on a mixed diet consisting of VitAlgae Nanno, RoBoost and fresh baker's yeast. At the end of the culture period, rotifers were enriched with Easy Dry Selco (EDS) to optimize their nutritional profile prior to feeding. Following enrichment, several microbial management strategies were evaluated. These strategies combined the use of a natural *Vibrio* controller (Sanocare SURE) with Sanolife MIC-F, which were applied either with or without a prior germination step, and at different concentrations ranging from 2×10^7 to 2×10^8 CFU ml⁻¹. In one treatment, *Bacillus* spp. were additionally administered on a daily basis directly in the culture tank at a concentration of 2×10^4 Bacilli ml⁻¹. To characterize the microbial community associated with the rotifers, bacterial plate counts were performed on a general culture medium (Tryptic Soy Agar (TSA)) and on a selective medium for *Vibrio* spp. (Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS)). The presence and growth of Bacilli were assessed after incubation of TSA plates for 24 h at 37°C. In parallel, rotifer samples were analyzed using Illumina-based 16S rRNA gene sequencing in order to determine the most abundant bacterial phyla.

Application in Artemia

EG SEP-Art® D-FENSE cysts were hatched and the *Artemia* nauplii were harvested after 21h, after which they were transferred to enrichment tanks. In one treatment, Sanolife MIC-F was added directly to the enrichment water after an appropriate germination step (1 h at 30°C), at a dosage of 1 g of germinated Sanolife MIC-F per liter of enrichment water. *Artemia* nauplii were then introduced into this matured water and subsequently enriched with EDS.

In additional treatments, enriched *Artemia* were first harvested, cleaned and concentrated before the addition of probiotics. Different dosages of germinated Bacilli were tested at varying nauplii concentrations, up to a maximum of 8 million nauplii.l⁻¹. The incorporation of the different Bacilli species into the *Artemia nauplii* was evaluated at different time intervals following administration. As for rotifers, microbial plate counts were carried out and 16S rRNA gene amplicon sequencing was performed to characterize the bacterial communities associated with the *Artemia* nauplii.

Results

The results indicate that the functional & beneficial *Bacillus* species, when combined with the appropriate use of bacteriostatic products during live food preparation, can be effectively bio-encapsulated in both rotifers and *Artemia*. Across all tested application strategies, a clear modulation of the microbial community was observed. In particular, all treatments led to a reduced presence of Proteobacteria in the live food, accompanied by an increased relative abundance of Firmicutes, including *Bacillus* spp.

The use of technologies controlling bacterial development in live food, specifically SURE for rotifers D-FENSE for *Artemia*, contributed to a reduction of *Vibrio* loads in the live food. This reduction appeared to facilitate the encapsulation of the beneficial *Bacillus* species resulting in a more stable microbiota associated with the live food. Multiple application routes proved successful, highlighting the possibility to adapt microbial management protocols to different operational conditions in hatcheries.

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Discussion

This study demonstrates that targeted microbial management strategies are effective tools for modulating the bacterial communities associated with rotifers and *Artemia* nauplii. Across all evaluated application scenarios, the combined use of technologies designed to control microbial development together with the application of selected *Bacillus* strains consistently reduced the relative abundance of Proteobacteria, including *Vibrio* spp., while promoting an enrichment of Firmicutes, particularly *Bacillus* spp. within the live food microbiota. The use of SURE and D-FENSE not only limited the proliferation of opportunistic bacteria but also enhanced the uptake and bio-encapsulation efficiency of the administered *Bacillus*. Effective incorporation of germinated *Bacillus* cells was achieved through both enrichment-water application and post-harvest treatment of live food, highlighting the flexibility and robustness of the proposed approaches. Overall, these results support the integration of selective microbial management strategies with optimized bioencapsulation protocols as a proactive approach to control live food microbiota, enhance biosecurity and promote more stable microbial conditions in marine larviculture, in line with previous findings on *Bacillus*-based microbial management.

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ZOOTECNICAL AND TRANSCRIPTOMIC PROFILE OF NON-EATING EURASIAN PERCH LARVAE

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Introduction

The onset of exogenous feeding is a critical phase in early fish larval development and is often associated with high mortality, posing challenges for aquaculture (Yúfera & Darias, 2007). In Eurasian perch (*Perca fluviatilis*), larvae show considerable variability in their ability to initiate foraging (Pepin et al., 2014), with some individuals feeding immediately (early eaters), others starting days later (late eaters), and some never feeding at all (non-eaters). The current study aimed at investigating the differences between eaters and non-eaters under optimal rearing conditions using a combination of zootechnical and transcriptomic approach.

Material and Methods

Six families were produced by crossing one female with one male, and larvae were reared until weaning. Starting at first feeding (5 days post-hatching, DPH), early eaters and non-eaters were manually selected and reared in separate tanks. Subsequent selections at 6 and 7 DPH enabled the identification of larvae that later initiated foraging (late eaters), thereby ensuring accurate classification of true non-eaters individuals. Total RNA was extracted from groups sampled at 6DPH, and sequenced (150 bp paired-end, 40M reads per library). Differentially expressed genes analysis was performed using DESeq2. Gene ontology enrichment (GO) was conducted with ShinyGO. Specific genes were identified for further RT-qPCR validation.

Results and Discussion

Results indicates that the failure in foraging was the primary cause of early mortality, accounting for up to 50% of larval deaths. Eaters were larger in both length and weight compared to non-eaters, indicating that body size may influence the ability to initiate foraging. RNA-seq analysis of larvae at 6 DPH identified 3,117 differentially expressed genes (DEGs; $\text{padj} < 0.01$, $\log_2\text{FC} > 1$). Principal component analysis revealed a strong separation between eaters and non-eaters along PC1, accounting for 86% of the variance. Functional annotation indicated that these DEGs are largely involved in DNA replication and cell cycle pathways. Notably, non-eaters showed down-regulation of genes associated with lipid metabolism (e.g., *dhcr24*, *sqle*, *sc5d*). Together, these results suggest that failure to initiate foraging is associated with growth and metabolic processes, reflecting differences in physiological condition during early larval stages. This study provides a first framework for understanding the biological basis of early foraging ability in fish larvae.

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BEYOND DIGESTIBILITY: A MULTI-TRAIT NUTRITIONAL ASSESSMENT OF BENTHIC COPEPODS AS LIVE FEED CANDIDATES

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Introduction

Copepods are increasingly explored as a source of live feed for larviculture, yet not all copepod species seem equally suitable. Previous studies have shown that digestibility differs strongly among copepod species, with potential consequences for larval growth and survival.

Benthic copepods offer practical advantages for larviculture, such as favourable fatty acid composition, resilience to environmental variation and short reproduction cycles, allowing for higher harvesting rates. However, adaptation to benthic habitats often involves the development of thicker cuticles to withstand coarse sediment, which may reduce digestibility for fish larvae.

Materials and methods

In this study, we developed an *in vitro* assay that allows quantitative evaluation of digestibility of multiple benthic copepod species using the enzymes trypsin and lipase. Experimental species were chosen based on culturability and their ecological relevance in the estuarine benthic food web. *Artemia franciscana* nauplii were included as a reference. Complementary analyses of total protein content were performed to broaden the nutritional assessment. In addition, the fatty acid composition of all species was analysed using gas chromatography coupled with mass spectrometry (GC-MS). Protein content, digestibility, and fatty acid data were integrated into a multi-trait ranking system to evaluate the overall nutritional quality of each species.

Results and discussion

Total protein content varied significantly among species (one-way ANOVA, $F_{4,10} = 32.93$, $p < 0.001$), with *M. littorale* reaching the highest protein content ($47.0 \pm 1.6\%$ dry matter) and *N. spinipes* the lowest ($21.7 \pm 3.5\%$). *In vitro* protein digestibility also differed significantly among species ($F_{4,18} = 11.41$, $p < 0.001$), with *A. franciscana* and *D. palustris* showing the highest digestibility and *N. spinipes* showing negligible digestibility, consistent with the absence of a systematic enzyme effect in this species. Notably, protein content and digestibility were not correlated: *M. littorale* combined a high protein content with one of the lowest digestibilities, indicating that a substantial fraction of its protein is not bioavailable to fish larvae, and that neither parameter alone is sufficient to rank species for nutritional quality. Fatty acid analysis (GC-MS) further showed that *T. discipes* and *M. littorale* were richest in DHA, while *D. palustris* contained the highest EPA levels; *N. spinipes* consistently ranked poorly across nutritional indicators. Integrating protein content, digestibility, and key fatty acid ratios (DHA:EPA, ARA:EPA, n-3:n-6, PUFA:SFA) into a multi-trait ranking revealed that no single copepod species performed favourably across all indicators, reflecting trade-offs between protein and lipid quality traits. These results highlight the added value of a multi-parameter approach for selecting suitable live feed candidates in marine fish larviculture.

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INNOVATIVE NON-PHARMACOLOGICAL APPROACHES FOR MANAGING *Caligus rogercresseyi*: IMPLICATIONS FOR THE HEALTH AND WELFARE OF ATLANTIC SALMON

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Salmon lice, *Lepeophtheirus salmonis* and *Caligus rogercresseyi*, represent a major management challenge in salmonid aquaculture, imposing substantial economic losses primarily through repeated pharmacological treatments and handling events that induce physiological stress, increase susceptibility to secondary infections, and compromise growth performance. The progressive depletion of effective antiparasitic treatments, driven by escalating resistance, environmental concerns, and animal welfare constraints causing economic losses, has created an urgent need for sustainable alternatives in salmon lice management.

This study examined the integrated botanical effects and host physiological responses of Rosseus Plus®, a phytogenix drinking supplement, administered via therapeutic baths and waterborne exposure against *Lepeophtheirus salmonis* and *Caligus rogercresseyi* under both controlled in vitro conditions and commercial-scale field trials in Atlantic salmon.

Controlled in vitro bioassays employing lice motility and responsiveness as early-stage screening endpoints, alongside field trials utilizing wellboat delivery, were conducted to characterize ectoparasitic burden dynamics across developmental stages. In vitro assays demonstrated 93–100% efficacy against adult *Caligus rogercresseyi* and *Lepeophtheirus salmonis*, respectively, while field trials employing achieved parasite reductions of 98% in adults and 85% in juveniles.

Molecular, histological, and physiological analyses collectively demonstrated systemic attenuation of acute and chronic stress responses, oxidative stress mitigation, and significant reduction of gill and skin inflammatory infiltration. Treated fish exhibited preserved neuroendocrine homeostasis, evidenced by stable cortisol profiles, maintained lysozyme activity, uncompromised innate immune function, balanced osmoregulation, and consistent serotonin concentrations. Waterborne administration of Rosseus Plus® potentiated innate immune responses, attenuated key stress biomarkers, and enhanced feed intake and growth performance. The integration of antioxidant, serotonergic, and immunological biomarkers within a comprehensive multi-parametric analytical framework established a scientifically rigorous and reproducible platform for precision monitoring of host resilience, welfare, and treatment outcomes in intensive aquaculture systems.

These findings support Rosseus Plus® as a safe, effective, and environmentally sustainable solution for integrated parasite management in Atlantic salmon aquaculture, promoting welfare-focused and responsible production practices.

A FULLY NON-PHARMACOLOGICAL STRATEGY FOR *Caligus rogercresseyi* CONTROL AND WELFARE IMPROVEMENT IN ATLANTIC SALMON

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Sea lice (*Caligus rogercresseyi*) represent one of the most significant health, welfare, and economic challenges in Atlantic salmon (*Salmo salar*) aquaculture in Chile. Conventional control strategies rely heavily on pharmacological treatments and repeated handling events, which may negatively affect fish welfare and production performance. This study evaluated the effectiveness of a fully non-pharmacological sea lice management strategy combining Aware Plus®, a phytogenic feed additive, and a single strategic Rosseus Plus® bath under commercial farming conditions throughout complete seawater production cycles. The strategy was implemented during 12-month seawater grow-out periods and assessed using welfare, health, production, and sustainability indicators. The integrated approach enabled 9–10 months of production without antiparasitic interventions, followed by a single non-pharmacological bath prior to harvest. Cumulative mortality remained below 5%, representing a 55% reduction compared with industry averages. Antiparasitic treatments were eliminated, antibiotic-associated disease transmission decreased by 49–100%, and fasting days were reduced by 99.1%. Fish achieved an additional harvest weight of 678–845 g per individual compared with conventional production benchmarks. Additional benefits included reduced melanosis, improved skin and mucus integrity, lower cataract incidence, and improved gill condition, indicating enhanced fish health and welfare throughout the production cycle. Environmental sustainability indicators showed zero chemical residues, the use of biodegradable inputs, and a 17–23% reduction in carbon deposition. These findings demonstrate that a fully non-pharmacological sea lice management strategy can successfully sustain a complete seawater production cycle while improving fish welfare, health, productivity, and environmental performance. The results support the integration of welfare-focused and sustainable management approaches as viable alternatives to conventional parasite control in Atlantic salmon aquaculture.

PHYTOGENIC SEA LICE MANAGEMENT IN SALMON: ECTOPARASITIC CONTROL AND NEUROENDOCRINE-IMMUNE-OXIDATIVE HOMEOSTASIS RESTORATION AS A SUSTAINABLE WELFARE STRATEGY

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Salmon lice, *Lepeophtheirus salmonis* and *Caligus rogercresseyi*, represent a major management challenge in salmonid aquaculture, imposing substantial economic losses primarily through repeated pharmacological treatments and handling events that induce physiological stress, increase susceptibility to secondary infections, and compromise growth performance. The progressive depletion of effective antiparasitic treatments, driven by escalating resistance, environmental concerns, and animal welfare constraints causing economic losses, has created an urgent need for sustainable alternatives in salmon lice management.

This study examined the integrated botanical effects and host physiological responses of Rosseus Plus®, a phytogenic drinking supplement, administered via therapeutic baths and waterborne exposure against *Lepeophtheirus salmonis* and *Caligus rogercresseyi* under both controlled *in vitro* conditions and commercial-scale field trials in Atlantic salmon.

Controlled *in vitro* bioassays employing lice motility and responsiveness as early-stage screening endpoints, alongside field trials utilizing wellboat delivery, were conducted to characterize ectoparasitic burden dynamics across developmental stages. *In vitro* assays demonstrated 93–100% efficacy against adult *Caligus rogercresseyi* and *Lepeophtheirus salmonis*, respectively, while field trials employing achieved parasite reductions of 98% in adults and 85% in juveniles.

Molecular, histological, and physiological analyses collectively demonstrated systemic attenuation of acute and chronic stress responses, oxidative stress mitigation, and significant reduction of gill and skin inflammatory infiltration. Treated fish exhibited preserved neuroendocrine homeostasis, evidenced by stable cortisol profiles, maintained lysozyme activity, uncompromised innate immune function, balanced osmoregulation, and consistent serotonin concentrations. Waterborne administration of Rosseus Plus® potentiated innate immune responses, attenuated key stress biomarkers, and enhanced feed intake and growth performance. The integration of antioxidant, serotonergic, and immunological biomarkers within a comprehensive multi-parametric analytical framework established a scientifically rigorous and reproducible platform for precision monitoring of host resilience, welfare, and treatment outcomes in intensive aquaculture systems.

These findings support Rosseus Plus® as a safe, effective, and environmentally sustainable solution for integrated parasite management in Atlantic salmon aquaculture, promoting welfare-focused and responsible production practices.

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. Sal 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 AqBiocare 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 take care the production and quality of the skin mucus, promoting the 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 an reduced skin damage compared with Formalin 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, AqBiocare Plus® increases mucous cell density in the skin, improve the quality and the quantity of the mucus, strengthening the fish's natural barrier, it has better effect on Lysozyme. Welfare analyses, including serotonin, cortisol, lactate, glucose and osmolality measurements, highlight its positive impact on fish well-being.

These comprehensive benefits position AqBiocare Plus® as an innovative and sustainable alternative in salmon farming, effectively protecting fish health, enhancing productivity and improving animal welfare.

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 septicemia (SRS), including CD4, CD8, IFN- γ , TNF, IL-1 β , 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.

DECIPHERING OSTREID HERPESVIRUS 1 ENTRY IN ITS HOSTS *Magallana gigas*: KEY ROLE OF THE MUCOSAL INTERFACE

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Introduction

The Pacific oyster *Magallana gigas* is the main farmed oyster species in France and worldwide, representing a major economic resource. However, intensive aquaculture practices and the introduction of exotic species have increased the risk of infectious diseases. Over recent decades, mass mortality events in juveniles have been associated with Ostreid herpesvirus 1 (OsHV-1) and have caused severe economic losses. Currently, no effective treatment exists, making disease prevention and health management critical for industry sustainability.

In bivalves, mucosal surfaces represent the first and most critical interface between the host and its environment, constituting a primary barrier against pathogen entry. Beyond its physical role, mucus is now recognized as a highly dynamic immunological compartment composed of a complex mixture of immune effector molecules and a diverse resident microbiota, considered as an integral component of host homeostasis. Despite this growing body of evidence, the role of mucus-associated immunity and microbiota in the context of OsHV-1 infection remains largely unexplored. In this context, we investigate the interactions between OsHV-1 and *M. gigas* at the mucosal interface, with the goal of identifying factors driving virulence and resistance. With this goal in mind, earliest host–pathogen interactions at the mucosal interface have been investigated through experimental OsHV-1 infections. Six different oyster lineages, produced in Ifremer facilities and expressing contrasted susceptibility to OsHV-1, have been used in order to understand whether mucosal immunity plays a role in this contrasted phenotypic response to viral infection.

Materials & Methods

Following pallial mucus collection according to time post infection, particular attention has been given to immune-related components, mucus-associated microbiota, and viral load dynamics at early stages of infection. Mucus samples have been analyzed using holistic approach, including: (i) proteomic characterization of immune-related proteins secreted in pallial mucus using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS); (ii) bacterial community profiling through 16S metabarcoding using Oxford Nanopore Technology sequencing; and (iii) viral load dynamics in pallial mucus over time post-infection.

Results

Oyster's survival rates confirmed the contrasting susceptibility between groups: low-susceptibility oysters (L) showed approximately 90% survival after one week, whereas highly susceptible oysters (H) exhibited less than 10% survival at 48 hours post-infection (hpi). Quantitative PCR analyses further revealed that viral load in pallial mucus increased significantly over time (two-way ANOVA, $p < 0.001$). Importantly, a significant interaction between time and oyster group was observed ($p = 0.0024$), indicating that viral load dynamics differed between L and H oyster families.

Moreover, preliminary analyses of the bacterial microbiota associated with pallial mucus revealed significant differences in both richness and diversity between L and H oyster families. In parallel, proteomic analyses identified more than 4,500 master proteins across all samples, including proteins involved in antiviral responses. Preliminary data visualization also suggested clear differences between oyster families.

Discussion

Ongoing analyses will provide further insight into the earliest mechanisms and interactions occurring at the mucosal interface between OsHV-1, host immune proteins secreted in mucus, and the associated bacterial microbiota. Furthermore, the generated datasets—including bioinformatic resources on mucus composition, microbiota, and responses to pathogen pressure—will be made accessible to the scientific community, fostering further research in marine biology, disease ecology, and environmental health.

PARENTAL SELENIUM NUTRITION FROM TUNA BY-PRODUCTS AND DIETARY MERCURY EXPOSURE: IMPLICATIONS FOR OFFSPRING PERFORMANCE IN RAINBOW TROUT *Oncorhynchus mykiss*

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Introduction

Broodstock nutrition is a key determinant of offspring quality in salmonids. Tuna by-products (TBP) represent a promising alternative to fishmeal due to their high selenium (Se) content. However, their potential contamination with mercury (Hg) raises concerns regarding possible transgenerational effects (Berntssen et al., 2004; Donnarumma et al., 2021). This study aimed to evaluate the respective effects of parental dietary Se supplied by TBP and dietary methylmercury (MeHg) exposure in offspring on growth performance in rainbow trout (*Oncorhynchus mykiss*) fry. In addition, the regulation of selenoproteins in response to both Se supply and Hg exposure was investigated using immunohistochemical approaches.

Materials and methods

Five broodstock diets were tested: a plant-based control (VEG), a conventional fishmeal-based diet (FM60), and three diets including TBP at 15% (TBP15), 30% (TBP30), and 60% (TBP60), providing Se levels ranging from 0.3 to 8 mg Se kg⁻¹ diet. Groups of 20 females and 10 males were reared at 7 ± 1 °C and fed the experimental diets for 6 months prior to spawning. Oocytes from each female were fertilized with milt from males within the same dietary group.

The resulting fry were reared at 17 ± 1 °C and fed for 3 weeks either a plant-based control (FCO) or MeHg contaminated diet (FHG, 2 mg Hg kg⁻¹ feed). At the end of each feeding period, zootechnical performance was assessed in broodstock (growth, reproductive success) and offspring (growth, feed efficiency, survival). Data were analysed by ANOVA and expressed as means ± SD.

Results

No significant differences in growth performance were observed among broodstock dietary groups. No significant interaction between parental diet and offspring diet was detected for the measured parameters.

In contrast, offspring fed the MeHg-contaminated diet (FHG) showed significantly lower specific growth rate (SGR) and higher feed conversion ratio (FCR) compared to those fed the control diet (FCO), while survival was unaffected (Fig. 1).

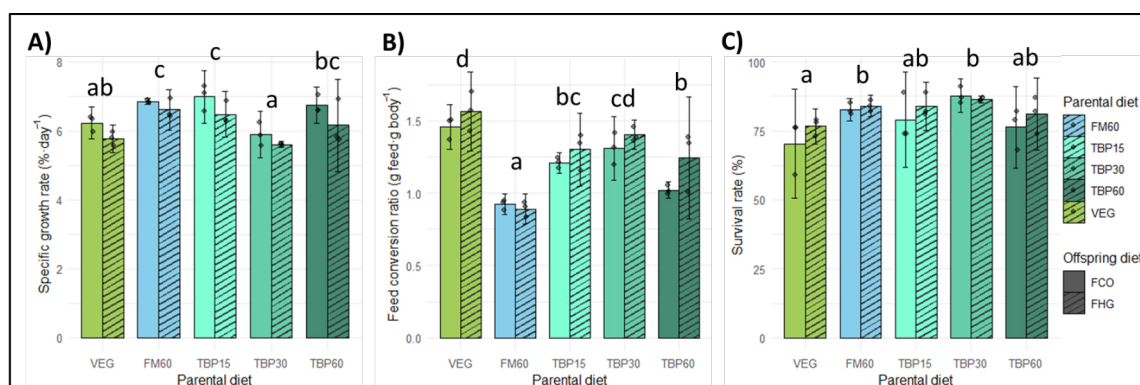


Fig. 1. A) Specific growth rate (% day⁻¹), B) Feed conversion ratio (FCR; g feed·g⁻¹ body mass), and C) Survival (%) as a function of parental diet and offspring diet. Bars represent mean values for each parental diet group (VEG, FM60, TBP15, TBP30, TBP60). Colours indicate parental diets while patterns distinguish offspring diets (FCO: solid; FHG: striped). Error bars represent standard deviation. Different letters indicate significant differences among parental dietary groups ($p < 0.05$).

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Parental diet influenced offspring performance: fry originating from VEG and TBP30 groups exhibited lower SGR compared to FM60 and TBP15 ($p < 0.05$). FCR was lowest in FM60 and highest in VEG ($p < 0.05$). Survival was higher in TBP30 and FM60 compared to VEG.

Discussion

No interaction between parental diet and offspring feeding regime was detected, suggesting limited influence of parental Se transfer on early-life Hg exposure under the present conditions. Dietary MeHg exposure impaired growth performance, as reflected by reduced SGR and increased FCR. Parental diet nevertheless modulated offspring performance, with TBP-based diets showing comparable or slightly lower performance than FM60, and no clear effect of dietary Se level on growth at this stage, possibly reflecting the specific chemical form of Se provided by tuna by-products, predominantly selenoneine, whose metabolism and transgenerational transfer remain largely unexplored.

Ongoing investigations aim to decipher the mechanistic basis of Se-Hg interactions, with a particular focus on selenoprotein regulation, tissue distribution, and oxidative stress pathways, providing key insights into the nutritional modulation of mercury toxicity during early development. These results will help refine the use of tuna by-products as a sustainable ingredient in aquafeeds.

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TECHNOLOGY ADOPTION BY SMALLHOLDER FISH

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Introduction

Aquaculture technologies, in terms of equipment and machinery, have supported the increasing productivity of aquaculture. However, the benefits of aquaculture technologies depend on the adoption by fish farmers. Previous studies revealed that the adoption level of management practices by smallholder fish farmers is low to moderate. Previous studies also found that farm and farmer characteristics were associated with the adoption of management practices among smallholder fish farmers (Islam et al., 2024; Muddassir et al., 2019; Susilo et al., 2019). Despite this, most studies have analyzed the adoption of management practices in aquaculture, but rather limited studies have studied the adoption of aquaculture technologies. Furthermore, smallholder fish farmers dominate global aquaculture and play an important role in generating household income and rural socioeconomic development (Aung et al., 2023; Mantey et al., 2020). Despite their importance, technology adoption among smallholder fish farmers remains under-researched. This study aimed to understand the adoption level and determine the variables associated with technology adoption among inland smallholder fish farmers.

Material and Method

Purposive sampling was used to select Tilapia fish farmers in Ciamis, Indonesia. Tilapia was the most produced inland aquaculture product in Indonesia. The survey was conducted by face-to-face interviews. Descriptive analysis was conducted on fish farmers' characteristics and aquaculture technology adoption. To identify the variables associated with the adoption of aquaculture technology, multinomial logistic regression analysis was employed based on (Agresti, 2013) 6, and 7, we modeled binary response variables with binomial GLMs. Multicategory responses use multinomial GLMs. In this chapter we generalize logistic regression to handle multinomial response variables, with separate models for nominal and ordinal cases. In Section 8.1 we present a model for nominal responses. It uses a separate binary logistic equation for each pair of response categories. In Section 8.2 we present a model for ordinal responses, using logits of cumulative response probabilities. In Section 8.3 we use other link functions for those cumulative probabilities and consider alternative ordinal logit models. In Section 8.4 we present tests of conditional independence with multinomial responses using models and using generalizations of the Cochran-Mantel-Haenszel statistic. In Section 8.5 we introduce a multinomial logit model for discrete-choice modeling of a subject's choice from one of several options when values of predictors may depend on the option. The final section discusses Bayesian methods for multinomial response modeling. 8.1 NOMINAL RESPONSES: BASELINE-CATEGORY LOGIT MODELS For a nominal-scale response variable Y with J categories, multicategory (also called polytomous. Moreover, backward selection was conducted to select the independent variables (Mendenhall & Sincich, 2012).

Result and Discussion

Overall, the adoption of aquaculture technology levels was low. Aerators (blowers), biofloc, and paddle wheels were adopted by about 20%, 14%, and 10% of farmers, respectively. Smart technologies such as water quality sensors, smart feeders, and aerators with oxygen sensors had lower adoption levels than previously mentioned technologies (1.5%, 0%, and 3.1%, respectively). This study revealed that fish farming experience, education, and subdistrict were significantly associated with the adoption of aquaculture technology. The findings of this study suggest that policies aimed at increasing technology adoption should account both farmers' capacities and local environmental conditions. Accordingly, the study recommends: (1) prioritizing government technology programs in the region with moderate to poor water quality, (2) improving access to financial resources, (2) strengthening knowledge dissemination, and (3) providing intensive training to support proper application of aquaculture technologies.

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Table 5. Result of multivariable multinomial logistic regression model: associated variables to aquaculture technology adoption (no adoption vs. dis-adoption vs. current adoption, n=196)

Variable and category	Dis-adoption vs No adoption			Current adoption vs No adoption		
	ME	Coef. (SE)	95% conf. interval	ME	Coef. (SE)	95% conf. interval
Experience	0.01	0.05 (0.02)**	0.01-0.09	0.001	0.02 (0.02)	-0.018 - 0.06
Education						
Elementary school	Ref. ^a			Ref. ^a		
High school	0.03 ^a	0.43 (0.59)	-0.74 - 1.60	0.04 ^a	0.45 (0.55)	-0.62-1.53
University	0.20 ^b	1.79 (0.68)**	0.46 - 3.11	0.08 ^a	1.16 (0.67)	-0.15 - 2.47
Subdistrict						
Kawali	Ref. ^a	Ref.		Ref. ^a		
Baregbeg	0.12 ^a	0.90 (0.82)	-0.70 - 2.51	-0.04 ^a	0.02 (0.68)	-1.30 - 1.35
Cijeungjing	0.09 ^a	0.95 (1.09)	-1.18 - 3.09	0.07 ^a	0.57 (0.84)	-1.08 - 2.22
Cikoneng	0.21 ^a	1.24 (0.97)	-0.66 - 3.14	-0.11 ^{a,c}	-0.29 (0.98)	-2.20 - 1.63
Cipaku	0.03 ^a	-0.06 (0.80)	-1.63 - 1.52	-0.19 ^{a,d}	-1.32 (0.72)	-2.73 - 0.09
Lumbung	0.03 ^a	0.14 (1.06)	-1.94 - 2.21	-0.06 ^{a,e}	-0.31 (0.85)	-1.99 - 1.36
Sadananya	-0.05 ^a	-0.97 (0.90)	-2.73 - 0.80	-0.20 ^{a,f}	-1.56 (0.71)*	-2.96 - -0.16
Sindangkasih	- 0.004 ^a	-0.48 (0.91)	-2.26 - 1.29	- 0.25 ^{b,c,d, e,f}	-2.41 (1.15)*	-4.67 - -0.16

Note: independent variable selection method: backward selection; marginal effect displayed was the average marginal effect (AME) from all observations; n = 196; all VIF values < 5; prob > Chi2 = 0.0039; McFadden's R2: 0.122; a, b, c, d, e, f: differ superscripts indicates differ value within column, **: significant at P-value < 0.01, *: significant at P-value < 0.05

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ECONOMIC PERFORMANCE OF WATER QUALITY TECHNOLOGY APPLICATION IN SMALLHOLDER FISH FARMERS

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Introduction

Water quality is a crucial factor impacting fish health in aquaculture (Akhter et al., 2021). Poor water quality limited stocking density, reduced fish growth, and increased mortality, which affect the aquaculture farm's economic performance. Technologies such as biofloc and aeration technologies were developed to improve water quality. However, knowledge of the association between technology and the economic farm performance of aquaculture is limited, especially among smallholder fish farmers. This paper aims to investigate the association between water quality improvement through technology application on the economic performance of Tilapia smallholder fish farmers.

Material and Method

Cross-sectional farm characteristics and economic data were collected in 2025 from 160 fish farmers in Ciamis, Indonesia, using a structured questionnaire. Moreover, production and economic indicators (revenue, costs, and partial gross margin) were calculated per m² per year. Descriptive statistics showed the difference between farms with and without technology, as assessed by the Mann-Whitney test. Additionally, the association of technology and farm characteristics on economic performance was investigated by a multiple linear regression model using Ordinary Least Squares (OLS) (Wooldridge et al., 2009).

Result and Discussion

The general farm characteristics resulted from 160 farmers participating in this study, consisting of 88% no-adopters, 9% paddle wheel adopters, and 3% biofloc adopters. Based on descriptive statistics, water quality technology might be associated with higher production and revenue. Increasing water quality leads to increasing fish health, which causes farmers to be more confident in increasing stocking density. Moreover, the higher stocking density achieved through technology application led to higher costs for fingerlings, feed, and electricity. In addition, the greater distribution of fish production among fish farmers using biofloc, compared to those using aeration technologies, might reflect huge differences in proficiency with biofloc technology among farmers. Multiple regression results showed that technology was not associated with the partial gross margin. The reason could be that relatively few farms adopted technologies. The other reason might be that increased production was not sufficient to cover rising production costs caused by the application of technology. Improving institutional support in terms of information, capacity building, and technical support was essential to enhance the adoption and effectiveness of water quality technology applications.

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EFFECTS OF FEEDING CONTEXT AND HEMPSEED MEAL ON AGGRESSIVE BEHAVIOUR IN NILE TILAPIA (*Oreochromis niloticus*)

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Introduction

Behavior interactions are a key component of social dynamics in group-reared fish and can significantly influence welfare and production efficiency (De Verdal et al., 2019). In Nile tilapia (*Oreochromis niloticus*), aggression is often associated with feeding events, yet quantitative data comparing feeding and non-feeding contexts under commercial tank conditions remain limited (Fattah et al., 2021). The increasing need to reduce reliance on soybean meal in aquafeeds has driven interest in more sustainable alternative plant ingredients, including hempseed meal derived from *Cannabis sativa*. Hempseed meal has been proposed as a potential partial substitute for conventional protein sources in aquafeeds due to its lower environmental footprint and favourable amino acid profile. Dietary composition can influence feeding dynamics and competition, potentially affecting social interactions in group reared fish (Ward et al., 2006). However, hempseed meal effects on behaviour in socially dynamic rearing systems remain largely unexplored. This study aimed to quantify aggressive behaviour across feeding and non-feeding periods over a six week trial and to assess whether dietary inclusion of hempseed meal influences aggression patterns in Nile tilapia.

Materials and Methods

Six 200 L glass tanks (control and hempseed meal diets; $n = 3$ per treatment) stocked with 28 Nile tilapia per tank were video recorded during weeks 2, 5, and 6; with three recording sessions per week during both morning and afternoon feeding events. All tanks were connected to the same recirculating aquaculture system (RAS). The hempseed diet was formulated by replacing 30% of the control diet with hempseed meal, while the control diet represented a standard commercial formulation. Video cameras were installed at the front of each tank and recordings of 2h were conducted from 1h before feeding started. Behaviour was quantified from 20 min video segments, with one segment analysed per tank, week, replicate, and context (feeding or non-feeding), resulting in 18 segments per tank ($n = 108$ total). Segments representing non-feeding and feeding contexts were derived from morning and afternoon recordings, respectively. Recordings were annotated using BORIS (Behavioural Observation Research Interactive Software; v9.7, Friard & Gamba, Italy), and aggressive acts were classified as nipping, chasing, lateral fights, and mouth fights. Aggression counts were analysed using a generalized linear mixed model (GLMM) with a negative binomial distribution and log link in IBM SPSS Statistics (v31, IBM Corp., USA).

Results

Feeding context strongly affected aggression, with lower counts of aggressive events across all behaviour types during feeding than non-feeding periods ($IRR = 0.55, p < 0.001$). Behaviour type explained most variation ($p < 0.001$), with chasing occurring approximately twice as frequently as nipping, while lateral and mouth fights were less frequent. Aggression declined across weeks ($p < 0.001$). Diet affected aggression ($p < 0.001$), with higher counts observed in the hempseed treatment compared to the control, but also higher final weight and improved feed conversion ratio compared to the control. Recording replicate had no significant effect ($p = 0.113$), and no diet $\times$ period interaction was detected ($p = 0.328$).

Discussion

The reduction in aggression during feeding suggests that fish prioritise feeding over social interactions, leading to a temporary suppression of aggressive behaviour (Montero et al., 2009). Differences among behaviour types indicate that low intensity interactions, such as chasing, dominate the aggression profile, while more energetically costly behaviours occur less frequently. The decline in aggression across weeks may reflect progressive social stabilisation within groups. Although diet affected overall aggression levels, the absence of a diet $\times$ period interaction indicates that dietary effects were consistent across feeding contexts and did not alter the behavioural response to feeding. The higher aggression observed in the hempseed treatment may reflect increased feeding motivation rather than a direct behavioural effect (Cutts et al., 2001; Peake & McGregor, 2004).

Acknowledgment

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WHOLE EGG POWDER AS A HIGHLY DIGESTIBLE SOURCE OF PROTEINS AND FATS IN JUVENILE ATLANTIC SALMON (*Salmo salar*)

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Whole egg powder represents a nutrient-dense and functionally versatile ingredient with potential relevance for the development of more sustainable aquafeed formulations. As aquaculture continues to expand, the sector faces increasing pressure to reduce its dependence on conventional marine and plant-based ingredient sources, particularly fishmeal and soy-derived ingredients, which can be associated with resource constraints, price volatility, land-use concerns, and environmental impacts. In this context, whole egg powder offers an alternative source of highly available protein, lipids, essential amino acids, phospholipids, cholesterol, vitamins, and bioactive compounds. ACTIPRO 44EP, a spray-dried whole egg powder, aims to support circular feed strategies by valorising high-quality animal-derived nutrients and reducing reliance on traditional raw materials. This study therefore evaluated the apparent digestibility and nutritional value of ACTIPRO 44EP in diets for juvenile Atlantic salmon (*Salmo salar*), with the objective of assessing its suitability as a high-quality alternative protein and fat source for salmonid aquafeeds.

The feeding trial followed a standard 70:30 digestibility model, in which the test diet was formulated with 30% ACTIPRO 44EP and 70% basal control diet. This approach allowed the digestibility of the egg powder ingredient itself to be calculated while maintaining comparability with published digestibility studies for aquafeed raw materials. Experimental diets were manufactured using a controlled extrusion process with a Clextral BC45 twin-screw extruder, producing stable 2–3 mm sinking pellets suitable for juvenile salmon. Juvenile Atlantic salmon (*Salmo salar*), with an initial mean body weight of 55.36 ± 1.21 g, were maintained in a freshwater recirculating aquaculture system (RAS) under controlled environmental conditions, including a water temperature of 15.20 ± 1.0 °C and continuous monitoring of dissolved oxygen, pH, and water quality. Fish were acclimated to the trial system and experimental diets before faecal collections began. During the digestibility phase, fish were fed to apparent satiation across three daily meals, and faeces were collected over a 42-day period using a consistent tank-level protocol designed to minimise nutrient leaching and avoid contamination with uneaten feed. Only fresh, intact, high-quality faecal material was retained for subsequent Apparent Digestibility Coefficient (ADC) analysis.

ACTIPRO 44EP demonstrated very high digestibility values for protein, energy, and individual amino acids in juvenile pre-smolt Atlantic salmon. The calculated ingredient ADC for crude protein was 96%, indicating efficient uptake and utilisation of nitrogen from the egg powder fraction. Essential amino acids also showed high apparent bioavailability, including lysine (94.53%), methionine (99.64%), and threonine (96.88%), which are central to protein deposition, growth, immune competence, and metabolic function in young salmon. In addition to the digestibility outcomes, fish fed the ACTIPRO 44EP diet showed numerically higher final individual body weights after 49 days compared with fish fed the control diet, although the digestibility design means growth performance should be interpreted as supportive rather than definitive. Throughout the trial, twice-daily clinical assessments confirmed that fish remained in good health, with no welfare concerns, abnormal faeces, or negative effects on water quality observed. Overall, the findings indicate that whole egg powder is a highly digestible and nutritionally valuable ingredient for juvenile Atlantic salmon and may represent a promising sustainable raw material for future salmonid feed formulations.

NEW APPROACH METHODOLOGIES (NAMs) FOR AQUACULTURE NUTRITION AND HEALTH

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Context

Currently, nutrition testing in aquaculture relies heavily on *in vivo* feeding trials, which involve raising fish or shrimp under controlled conditions and monitoring growth, feed utilization, immune and health-related criteria. These trials are resource-intensive, time-consuming (weeks to months), and require large numbers of animals, making them costly and ethically challenging. New Approach Methodologies (NAMs) offer an opportunity to modernise aquatic nutrition by integrating mechanistic, exposure relevant and species-specific information within a framework of validated contexts of use (CoU). NAMs include a variety of technologies, methodologies, and approaches such as *in vitro* tests, *in silico* computational models, biomarkers, and modified *in vivo* assays that can fill critical information gaps and build confidence for their use in proof of efficacy and regulatory decisions.

The availability of NAMs that adequately reflect fish biology remains a key gap in aquaculture research. While *in vitro* cell culture approaches and advanced platforms such as organoids (3D miniaturised organ-like structures derived from stem cells) and organ-on-chip systems (microfluidic devices mimicking organ functions) offer clear potential as NAMs, their application in fish and shrimp remains incipient. In this context, *ex vivo* fish tissue explants provide a pragmatic and biologically relevant alternative, preserving native tissue organisation and metabolic competence while supporting the principles of Replacement, Reduction and Refinement (3Rs) in animal nutrition research.

Methodological approaches

Explants are small sections of native tissue (e.g., intestine, skin, gills, scales) maintained *ex vivo* for short-term experiments. Explants preserve native tissue architecture and microbiome, cell-cell interactions, and biochemical signalling, offering a more physiologically relevant model than traditional cell lines. The *ex vivo* tissue explants approach bridges the gap between *in vitro* cell culture and whole-animal *in vivo* trials, enabling mechanistic insights into nutrient absorption, barrier function, and immune responses without the complexity of entire organisms.

In higher vertebrates, intestinal mucus is also increasingly used as a key component in NAMs, to simulate the gastrointestinal barrier for testing nutrient/drug absorption, toxicity, and host pathogen interactions. Additionally, fish and shrimp faeces are also a matrix with the potential to harbour a series of biomarkers linked to intestinal inflammation and immune status.

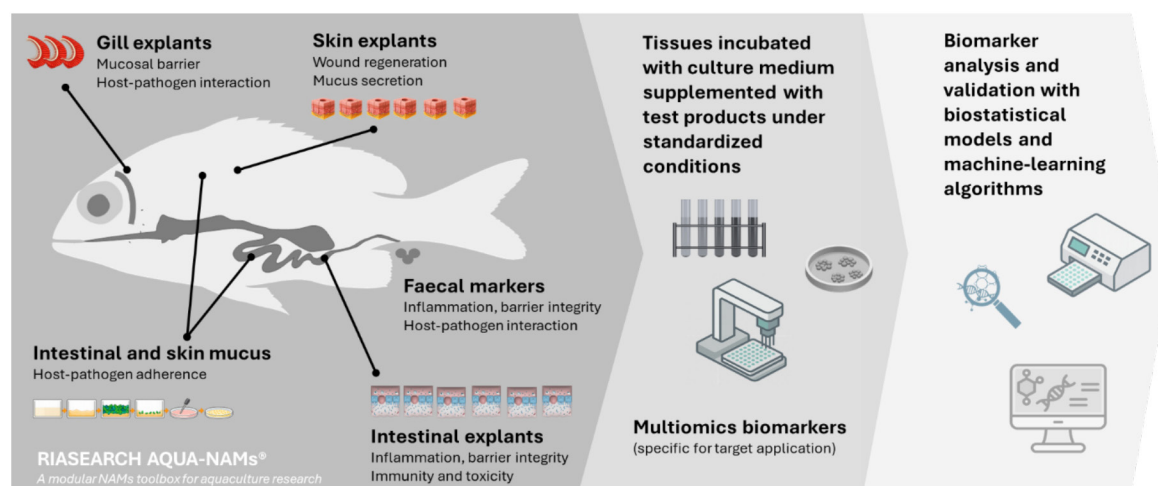


Figure 1. A general view of the RIASEARCH AQUA-NAMs platform.

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Results

While presenting a schematic roadmap of New Approach Methodologies (NAMs) in aquaculture species is conceptually straightforward, their practical implementation is extremely challenging, requiring a lengthy setup of novel methodologies, the identification and qualification of context-specific biomarkers, and the generation and integration of large, high-quality datasets to robustly establish *in vivo* relevance. We are actively engaged in this process, with established experimental platforms using intestinal and skin explants and mucus host-pathogen interaction assays across Atlantic salmon, Nile tilapia and European seabass. These systems are currently being applied to evaluate raw materials, feed additives and other non-nutritional compounds, integrating multiparametric endpoint frameworks encompassing immune response, epithelial integrity, and wound healing process. A selection of results derived from these ongoing efforts will be presented to exemplify the translational potential of NAMs in aquaculture nutrition research.

Conclusions

A NAMs-based approach is highly innovative, delivering faster and ethically sound testing options while providing mechanistic insights that *in vitro* tests cannot achieve. Although, at this stage, we are still far from fully replacing classical *in vivo* efficacy and tolerance trials, NAMs can transform early-stage nutritional screening, offering a significant competitive advantage in the aquafeed innovation pipeline.

Acknowledgment

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FROM BIOREFINERY TO SEA CAGE: *EX VIVO*-TO-FIELD VALIDATION OF ALGAE-DERIVED FUNCTIONAL ADDITIVES FOR MUCOSAL HEALTH AND WOUND HEALING IN ATLANTIC SALMON

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Introduction

Much of the production lost in European salmonid farming originates at the mucosal barriers: sea lice infestation, skin ulceration and delayed wound healing after handling and delousing, and multifactorial gill disorders. Functional feeds that reinforce skin-, gill- and gut-associated lymphoid tissue (SALT, GIALT, GALT) are one of the preventive strategies available to farmers, and algae are a potential source of the required bioactives — but the sustainability of the ingredient now matters as much as its function. The Horizon Europe project LOCALITY cultivates micro- and macroalgae on nutrient-rich industrial side streams and North and Baltic Sea waters and processes the biomass through a cascading biorefinery into fractions for the food, aquafeed, agriculture and textile markets.

In the aquaculture application arm of the project, it faces a problem common to all biorefinery-driven ingredient development: the biorefinery delivers many more candidate fractions than can realistically be tested *in vivo*, while each trial is expensive, slow and animal-intensive. The LOCALITY answers this with a three-tier de-risking funnel — *ex vivo* screening, controlled challenge validation, and commercial-scale field validation (Fig. 1) — designed so that only candidates with a mechanistic rationale reach animals, and only candidates that indicate improvement during a controlled challenge reach a sea cage.

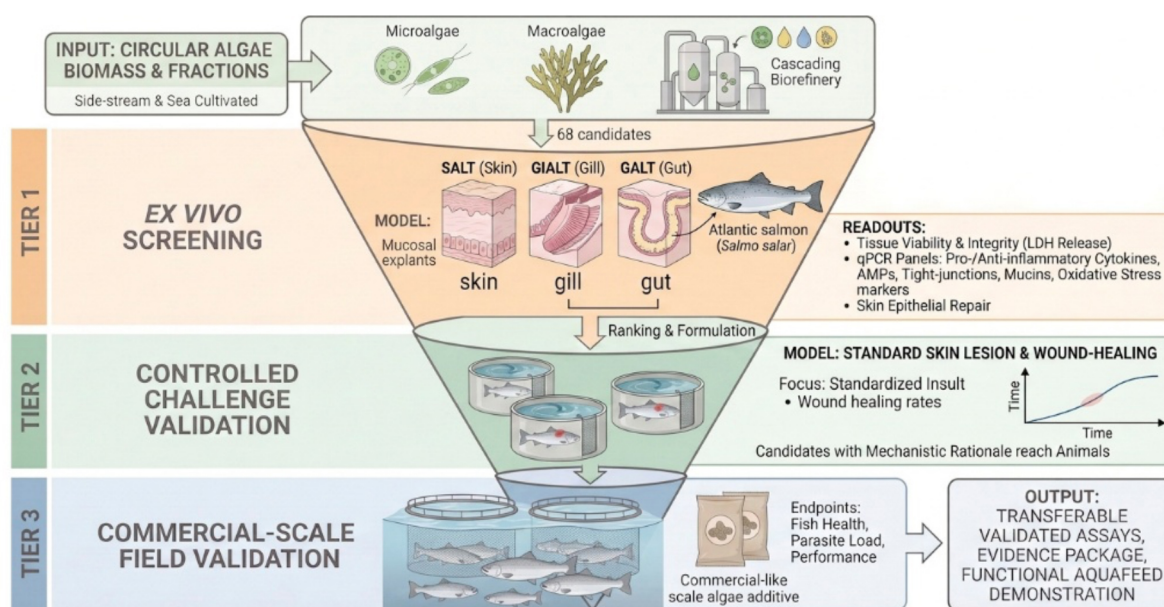


Figure 1. Overall structure of LOCALITY aquaculture application. Algae biomass and biorefinery fractions produced in the project enter a tiered pipeline: *ex vivo* screening of a candidate library against mucosal targets, functional validation of selected prototypes in controlled wound-healing models, and field validation of a commercial-like scale algae-based additive.

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Materials and methods

A candidate library of 68 algae biomasses and biorefinery fractions supplied by LOCALITY project (including seaweeds such as *Ascophyllum nodosum* and microalgae such as *Phaeodactylum tricornutum*) were characterised for proximate and bioactive composition and assembled into a screening library. In tier 1, *ex vivo* screening with mucosal explants (SALT, GIALT and GALT) were obtained from Atlantic salmon (*Salmo salar*) and cultured in 48-well plates using a one-animal-per-row design (16 explants per animal, 3 animals per plate). Explants were exposed to graded doses of each candidate, with reference agonists (β -glucan, LPS, poly I:C) and vehicle as controls. Readouts were tissue viability and integrity (LDH release) and qPCR panels covering pro- and anti-inflammatory cytokines, antimicrobial peptides, tight-junction proteins, mucins and oxidative stress markers. SALT defensive properties were assessed as epithelial repair after a standardised insult. Candidates were ranked by a composite score combining viability, immunomodulatory amplitude and barrier response, and the highest ranked were formulated into extruded prototype diets.

In the second tier functional validation was performed when selected prototypes were tested *in vivo* as promoters for wound healing after a standardised skin lesion. In the last tier a field validation takes place when the best-performing prototype is produced at commercial scale and validated on farm, with fish health assessment, parasite load and performance as endpoints.

Discussion and impact

The value of this work lies less in any single candidate than in the decision architecture around it. Screening on mucosal tissue rather than cell lines preserves the epithelium–immune cell–mucus context in which a feed additive has to work, while using a fraction of the animals a comparable *in vivo* screen would require — a direct 3Rs gain, and a realistic way to interrogate a biorefinery output space of this size. The tiered design front-loads failure when cytotoxic, inert or mechanistically implausible candidates are eliminated before diet extrusion, so trial capacity is spent on prototypes with a defensible rationale. *Ex vivo* responsiveness is a necessary but not sufficient condition for *in vivo* efficacy, and correlation between explant readouts and trial outcomes is being established prospectively through paired screening and challenge trials to further support interpretation.

For the sector, the aquaculture application of LOCALITY delivers three transferable outputs: a validated set of mucosal *ex vivo* assays applicable to other species and additive classes; a mechanistic evidence package of the type increasingly required for feed additive dossiers and sustainability claims, generated under a traceable circular value chain, and a demonstration that side-stream-cultivated algae can meet functional feed specifications at commercial scale. Results are communicated to feed producers, farmers and regulators through the LOCALITY dissemination programme and the Horizon Europe Sustainable Aquaculture project cluster.

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COMPARATIVE ANTIMICROBIAL RESISTANCE PROFILES OF BACTERIA ISOLATED FROM BALTIC SALMON AND SEA TROUT SPAWNERS

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Introduction

The health status of returning spawners can affect both natural recruitment and the quality of juveniles produced for restocking. Antimicrobial-resistant bacteria in aquatic environments are also relevant to the One Health framework because wild fish, hatchery operations and receiving waters may facilitate the spread of resistant bacteria and resistance determinants. This study characterised the bacterial genera isolated from Baltic salmon (*Salmo salar*) and sea trout (*Salmo trutta*) spawners from the Venta River, Latvia, compared their antimicrobial resistance profiles and examined whether resistance occurrence differed between wild-origin and hatchery-origin spawners.

Materials and Methods

During the 2023 and 2024 spawning seasons, 49 salmonid spawners (26 Baltic salmon and 23 sea trout), including wild-origin and fin-clipped hatchery-origin fish, were examined. In total, 190 samples were collected from gills, skin mucus, urogenital openings, lesions, reproductive material, eggs at different developmental stages and, when available, internal organs. Bacteria were cultured on non-selective and selective media and identified by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. Minimum inhibitory concentrations (MIC) were determined by broth microdilution for *Aeromonas* spp. (n = 62), *Pseudomonas* spp. (n = 62), *Shewanella* spp. (n = 40) and *Flavobacterium* spp. (n = 40). In addition, 76 *Aeromonas* isolates were tested against six antimicrobial agents by disc diffusion.

Results

Aeromonas spp. were detected in 125 of 190 samples (65.8%), *Pseudomonas* spp. in 93 (48.9%), *Shewanella* spp. in 74 (38.9%) and *Flavobacterium* spp. in 53 (27.9%). From several samples more than one genus was recovered. The genera showed distinct antimicrobial resistance patterns. By microdilution, *Aeromonas* isolates most frequently showed resistance to ampicillin (98.4%) and sulfadimethoxazole (72.6%), whereas no resistance was observed to ciprofloxacin or nalidixic acid. *Pseudomonas* isolates showed 100% resistance to ampicillin, cefotaxime and trimethoprim and more than 90% resistance to azithromycin, chloramphenicol and sulfadimethoxazole. In *Shewanella*, the highest resistance occurred to sulfadimethoxazole (80.0%) and ampicillin (40.0%), while resistance to the other evaluated agents did not exceed 10.0%. *Flavobacterium* isolates showed 100% resistance to trimethoprim, ceftazidime, gentamicin and cefotaxime, and 95.0% resistance to azithromycin. The descriptive comparison did not reveal a clear difference in the occurrence of resistant bacteria between wild-origin and hatchery-origin spawners.

Discussion and Conclusions

The high occurrence of several opportunistic or potentially pathogenic bacterial genera, together with genus-specific resistance profiles, supports inclusion of multiple indicator groups in salmonid health and antimicrobial resistance surveillance. The similar findings in wild-origin and hatchery-origin fish after their return to the same river environment suggest that shared environmental exposure may be important. Together, the results show that *Aeromonas* represents one informative component of a wider bacterial community with heterogeneous resistance patterns. Because validated clinical breakpoints are unavailable for some aquatic bacterial species and antimicrobials tested, the resistance results should be interpreted with caution. Continued standardised monitoring and susceptibility-guided treatment decisions are recommended for fish health management and responsible antimicrobial use.

Acknowledgment

This research was financially supported by the Ministry of Agriculture of the Republic of Latvia within the framework of the 2023–2024 study “Antimicrobial Resistance of Significant Microorganisms and Commensal (Indicator) Bacteria in Baltic Salmon (*Salmo salar*) and Sea Trout (*Salmo trutta*) Spawners in Latvia”.

VALIDATING AND APPLYING FECAL SWABS AS LESS-INVASIVE SAMPLING METHOD TO MEASURE CORTISIL LEVEL ON ATLANTIC SALMON COMMERCIAL SEA CAGES

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Introduction

Convenient sampling methods for non-invasive stress monitoring are needed to support welfare assessment in modern salmon aquaculture. Fecal samples have been validated and used for measuring stress levels of Atlantic salmon (*Salmo salar*). The fecal sampling method in the field is usually conducted by stripping fish or collected fecal material after dissecting fish in commercial sea cages (Cao et al., 2017). In the present study, we developed and validated a fecal swab method for quantifying cortisol in Atlantic salmon under commercial farming conditions. FLOQSwabs® Minitip were evaluated across six validation components: swab material uptake, field and laboratory comparability, biological validation, analytical validation, and storage stability.

Materials and Methods

Atlantic salmon were sampled from commercial sea-cage sites in Norway. All sampling was performed during routine operational procedures, including sea-lice counting and regular health check. All procedures involving fish handling and sampling followed Directive 2010/63/EU and complied with Norwegian regulations governing the use of animals in scientific research.

Blood samples were collected from the caudal vein using 4 mL heparinized BD Vacutainer® tubes (Becton, Dickinson and Co., NJ, USA). Fecal samples were firstly collected by inserting a sterile Mini Copan FLOQSwabs® (Cambio, Cambridge, UK), through the anus until the breakpoint of the swabs, and gently rotating the swab five times along the mucosal surface. This approach ensures sampling of both feces and gut mucosa. According to Ruiz et al., (2024), this method does not elicit abnormal behavior or visible signs of stress or injury in Atlantic salmon. Additional fecal material was collected by gently applying pressure along the abdomen to expel feces through the vent. The expelled fecal matter was transferred into sterile 2 mL tubes, homogenized thoroughly and stored at –80 °C before further analysis.

Plasma and fecal cortisol concentrations were determined using a commercial Neogen cortisol ELISA kit, following extraction and analysis procedures adapted from Ding et al., (2024).

Results

Swab could sample enough material for fecal cortisol analysis. The weight of material collection by one swab was consistent across three different fecal textures (mean 0.118 g ± 0.01 g; CV = 8.23%; p = 0.609). Field swabs produced cortisol concentrations comparable to laboratory resampling (paired p > 0.05) with a strong correlation (r = 0.94). Biological validation across three different commercial sites with four net pens in Norway demonstrated significant associations between plasma and fecal cortisol among sample groups (mixed-effects model: p = 0.044; Spearman's ρ = 0.67, p < 0.001). Significant increased cortisol levels were detected by fecal swabs after fish were posted to acute handling stress. In addition, baseline fecal cortisol concentrations differed among net pens, indicating that the method is sensitive enough to detect variation associated with different farming environments. Spike-and-recovery tests showed an overall recovery of 90.5% (range 62.9–127.5%). Repeatability testing of five homogenised fecal samples yielded a mean technical CV of 10.23%. Storage stability analysis indicated rapid degradation at room temperature, stable cortisol profiles at 4 °C for up to 14 days, and high stability at –20 °C for at least 60 days.

Summary

Overall, the fecal swab method provides a practical, non-invasive, and robust tool for stress hormone monitoring in farmed salmon. This approach offers strong potential for integration into routine welfare assessments and operational decision support in commercial aquaculture.

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TRAINING THE AQUACULTURE PROFESSIONALS OF TOMORROW: INNOVATIVE APPROACHES TO EDUCATION AND LIFELONG LEARNING

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Aquaculture is increasingly recognized as a key component of sustainable food production and the blue economy, creating a growing demand for professionals with interdisciplinary knowledge, practical skills, entrepreneurial competencies, and the ability to respond to technological and environmental change. Meeting these needs requires education and training that extends beyond traditional classroom learning and supports continuous professional development.

The University of Gdańsk (Poland), together with international partners through two EU-funded projects: AquaLoop (Interreg South Baltic) and Blue Bio Techpreneurs (EMFAF), has developed innovative educational approaches linking higher education, practical aquaculture training, entrepreneurship, and lifelong learning. These initiatives provide students and early-career professionals with opportunities to address real-world challenges related to sustainable aquaculture, circular production systems, and the blue bioeconomy.

A central element is challenge-based and experiential learning, enabling participants to connect theoretical knowledge with practical applications. Within the AquaLoop cross-border student exchange programme, students undertake pilot-scale laboratory activities and study visits focused on sustainable and circular aquatic production, including aquaculture and aquaponics. These experiences develop practical competencies in resource efficiency, circularity, environmental sustainability, and innovation.

International and interdisciplinary collaboration further strengthens the training model. Students from universities in Gdańsk, Klaipėda, and Rostock work in diverse teams, combining expertise in aquaculture, marine biology, environmental sciences, biotechnology, and related fields. This approach encourages knowledge exchange and develops the collaborative and problem-solving skills needed for a sustainable and internationally connected aquaculture sector.

The Blue Bio Techpreneurs (BBT) initiative complements this approach through entrepreneurship and innovation-oriented learning. During BBT Hackathons, students work in international, interdisciplinary teams to address real-world challenges in the blue bioeconomy and develop innovative solutions for sustainable aquatic production. Methods including design thinking, innovation management, mentoring, and stakeholder engagement help participants translate scientific knowledge into practical solutions while strengthening creativity, teamwork, leadership, communication, and problem-solving skills.

Digital education and lifelong learning provide an additional dimension. The BBT Massive Open Online Course (MOOC), comprising five modules available through the FutureLearn platform, offers flexible learning opportunities for students, early-career professionals, and other learners seeking to develop blue economy competencies. Such digital formats support continuous upskilling and reskilling as aquaculture technologies, production systems, markets, and sustainability requirements evolve.

Together, AquaLoop and BBT demonstrate how experiential learning, international collaboration, entrepreneurship, digital education, and lifelong learning can be integrated into aquaculture training. The experience of the University of Gdańsk provides a transferable model for universities, training providers, and aquaculture stakeholders seeking to develop a skilled, innovative, and sustainability-oriented workforce capable of meeting the future needs of the aquaculture sector.

INTEGRATED PRODUCTION OF *Holothuria arguinensis* AND MACROALGAE (*Ulva sp.* e *Gracilaria sp.*) AS BIOREMEDIATORS OF ORGANIC WASTE FROM MARINE FISH

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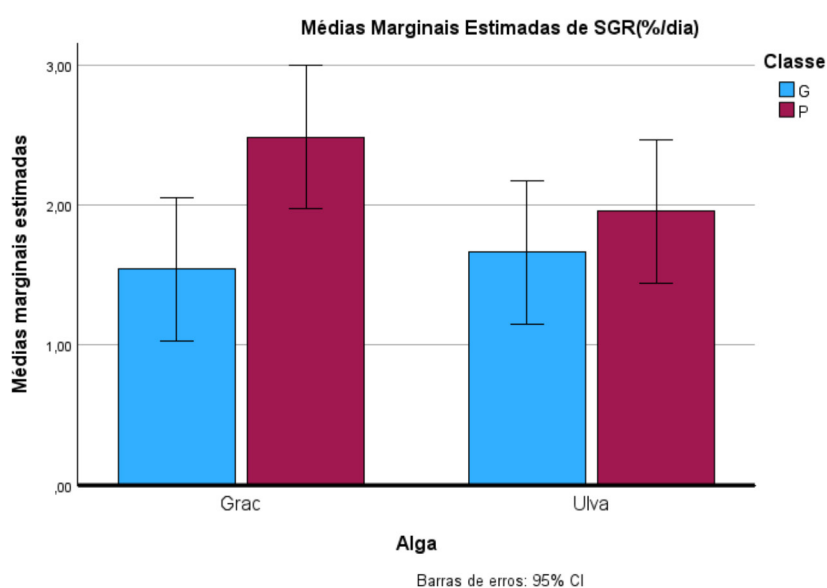
Introduction

Aquaculture plays a key role in global protein supply and in reducing pressure on wild fish stocks, but it faces sustainability challenges, particularly the release of nutrient-rich effluents that can lead to environmental degradation (FAO, 2022; Naylor et al., 2021). To address these issues, innovative approaches such as Recirculating Aquaculture Systems (RAS) and Integrated Multi-Trophic Aquaculture (IMTA) have been developed to improve resource efficiency and waste reuse (Granada et al., 2016; Neori et al., 2004). In this context, macroalgae such as *Ulva sp.* and *Gracilaria sp.* stand out for their ability to remove nutrients and generate economic value (Abreu et al., 2011; Bolton et al., 2009), while detritivorous species like *Holothuria arguinensis* contribute to organic matter recycling (Purcell et al., 2016; Zamora et al., 2016). The integration of these organisms within IMTA systems represents a promising strategy to enhance the environmental and economic sustainability of aquaculture (Chopin et al., 2001; Troell et al., 2009).

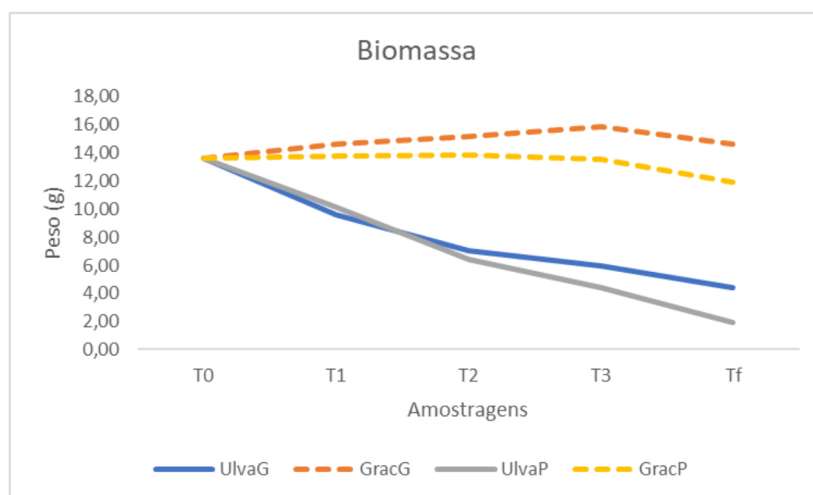
Materials and Methods

The experiment was conducted in a recirculating aquaculture system (RAS) integrated with a two-trophic level IMTA setup to evaluate the combined performance of macroalgae (*Ulva sp.* and *Gracilaria sp.*) and sea cucumbers (*Holothuria arguinensis*) in nutrient removal and biomass production. Four treatments were established based on macroalgae species and sea cucumber size classes (small: 0.5–2.0 g; large: 2.1–5.5 g), each in triplicate, totaling 12 experimental units. The trial lasted 51 days under controlled conditions (20 ± 1 °C; 12:12 h photoperiod). Macroalgae were cultured in photobioreactors, while sea cucumbers were maintained in tanks and fed regularly based on biomass (12% weekly). Water quality parameters (temperature, salinity, dissolved oxygen, ammonia, nitrite and phosphate) were monitored periodically. Growth performance of both organisms and nutrient removal efficiency were assessed. Statistical analysis was performed using analysis of variance (ANOVA).

Results



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An ANOVA was conducted to assess the effect of algal type and class on specific growth rate (SGR) of *Holothuria arguinesis*. The results revealed a significant effect of class on SGR ($p = 0.023$), indicating differences between the large (G) and small (P) groups. On the other hand, no significant differences were found associated with the alga factor ($p = 0.381$) or with the interaction between alga and class ($p = 0.178$). Levene's test was not significant ($p = 0.140$), confirming the homogeneity of variances.

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Changes in macroalgal weight across multiple sampling points (T0–TF). *Gracilaria sp.* showed growth, while *Ulva sp.* did not. The best results were observed in GracG, followed by GracP. Biomass loss was observed in UlvaG and UlvaP, with the loss being more pronounced in UlvaP.

Discussion

The results show that there were significant differences in SGR among the different classes. The small class (P) exhibited a higher SGR than the large class (G), indicating that the small class (P) grew better. This can be explained by the fact that smaller cucumbers require more protein for their growth. The associated conditions also contributed to the growth of these plants. It was evident that the type of algae did not affect the growth of the cucumbers, or vice versa. The feeding habits of the cucumbers would not be affected by the algae, as these organisms feed on organic matter deposited on the bottom. Growth is due to water parameters, with temperature being a critical factor in development. According to Madruga A. (2021), individuals cultured at a temperature of 20°C showed a better SGR of 0.50% day⁻¹ and in this study reached an SGR of 2.49% day⁻¹.

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SEASONAL VARIATION IN GILL HISTOPATHOLOGY OF COMMON CARP (*Cyprinus carpio*) REARED IN A SEMI-INTENSIVE PRODUCTION SYSTEM

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Introduction

Histopathological changes in fish are recognised as biomarkers of environmental stress. While some changes represent adaptive responses that help fish cope with natural fluctuations in environmental conditions, others may indicate deteriorating water quality, environmental contaminants, the presence of parasites, or disease. Gills are especially good biological indicators due to their continuous interaction with the environment and are among the first organs to exhibit histopathological alterations in response to change in the aquatic environment (Pramnik & Biswas, 2024). However, these markers are seldom used in aquaculture settings.

Materials and Methods

Sample collection for this study was conducted in 2024 during the whole production season, covering fish from one-year-old common carp to market-ready individuals. Samples were obtained from the Srpski Miletić fish farm, located in the northern part of Serbia. This fish pond receives its water supply from the Danube–Tisza–Danube (DTD) canal. Some of the most important purposes of the DTD canal are the drainage of agricultural land and the irrigation of arid areas. Sampling was performed during three periods: spring (26 April), summer (23 July), and autumn (06 December). Fish were anaesthetised, the second gill arch was dissected, fixed in formalin, processed and stained using a combination of haematoxylin and eosin. Each sample was scored from 0 to 3 to assess the extent of tissue alterations. A score of 0 indicated no detectable change, while a score of 3 indicated that most of the sample was affected by the pathological alterations. Statistical analyses were conducted using the non-parametric Kruskal–Wallis test.

Results

In general, the highest scores in the gills of common carp were found for circulatory disturbances (such as hyperaemia and stasis) and hyperplasia. Statistical analysis revealed significant seasonal differences ($p < 0.05$) for eight histopathological changes (Tab 1). Post-hoc analysis showed that most differences occurred between summer and either spring or autumn, while differences between spring and autumn were observed only for one alteration.

Discussion

Seasonal variation in gill histopathology was evident, with different histopathological profiles during each season, but without a specific pattern that could be clearly attributed to particular factors. In summer, higher scores for the occurrence of parasites coincided with hypertrophy of the respiratory epithelium, but it is also notable that scores for hyperplasia were lower compared with both spring and autumn. Hyperplastic changes as an adaptation to the presence of parasites are among the most frequent mechanisms in gills (Dezfuli et al. 2023). However, hyperplastic changes observed here are probably more related to environmental stressors during summer than to the presence of parasites. This is further corroborated by low scores of necrosis and low presence of EGCs, which are not actively engaged in the inflammatory process, in contrast to parasite infection (Dezfuli et al. 2011). Cyprinids have a specific mechanism in the gills, and in normoxic water at lower temperatures the gills lack protruding lamellae (Sollid and Nillson 2006), which is in contrast to other fish and explains why hyperplasia is present in spring and autumn. These findings support the use of gill histopathology as a useful biomarker for detecting seasonal variations in water quality in freshwater pond culture systems.

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Acknowledgment

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CONSUMER PERCEPTION AND ACCEPTANCE OF PROCESSED ANIMAL PROTEINS IN AQUAFEEDS: EVIDENCE FROM ITALY

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Introduction and methodology

In recent years, the search for alternatives to fishmeal (FM) and soybean meal (SBM) has highlighted several promising ingredients, including single-cell proteins (SCPs), algae and microalgae, and insect meals, which can support feed efficiency, fish welfare, and health (Serra et al., 2024). However, their high cost may slow adoption in a sector where feed accounts for 30–60% of total production costs (Mulazzani et al., 2021). In this context, processed animal proteins (PAPs) obtained from livestock by-products, particularly poultry- and porcine-derived PAPs, represent a potentially scalable and circular solution. These ingredients can offer a competitive alternative to FM and SBM in terms of growth performance and cost (Wilkinson & Young, 2020). However, less is known about how consumers perceive them. Although insect PAPs have been widely studied, poultry and porcine PAPs remain under-researched in consumer studies. To fill this gap, the present study examines consumer attitudes toward farmed fish fed diets containing poultry and porcine PAPs, using insect-derived PAPs as a point of comparison. Specifically, it addresses the following research questions: (i) what perceptions act as barriers to consumers' willingness to consume fish raised on PAP-based diets?; (ii) to what extent are consumers willing to pay (WTP) a premium for fish raised on PAP-based diets?; (iii) and does the provision of sustainability-related information influence that WTP?

The analysis is based on an online panel survey of 1,502 consumers living in Italy, selected through stratified sampling by gender, age, and region of residence. First, respondents' perceived naturalness of the wild fish diet was assessed using a multiple-choice question to contextualize their subsequent answers. Next, perceptions of conventional ingredients (FM and SBM) and PAPs were then elicited through open-ended questions and examined using sentiment analysis. Responses were classified as positive, negative, or neutral with an Italian-language model based on the *pysentimiento* Python library. WTC for fish reared on PAP-based diets and its main barriers were measured using seven-point Likert-scale statements covering four dimensions that emerged from the literature as having a direct effect: disgust, social norms, comparison with conventionally fed fish, and perceived product safety. These items were analyzed using a random forest (RF) classifier, with WTC as the outcome variable and the barriers as predictors. As an ensemble classification method that combines multiple decision trees, RF is well-suited for modeling consumers' WTC and identifying the relative importance of the barriers that shape their decisions. WTP was measured on a percentage scale with a midpoint for no price difference, and values above or below indicating a premium or a discount. Finally, to test the role of information, respondents were shown a short objective text on the sustainability of PAP-based feeds, and WTP was elicited again using the same scale. Changes in WTP were evaluated through a paired pre-post design using the Wilcoxon signed-rank test.

Results and discussion

Results show that 83% of consumers could identify at least one possible component of the natural diet of fish. Plant-based organisms (59%), fish of other species (41%), and insects (31%) were perceived as more closely aligned with the diet of wild fish, whereas small terrestrial animals, such as mammals and reptiles (16%), were considered less likely to be part of it. Sentiment analysis revealed predominantly neutral attitudes toward FM and SBM. By contrast, insect, poultry, and porcine PAPs elicited polarized reactions in about half of the sample (43–49%). Among respondents expressing either positive or negative sentiment, insect PAPs were evaluated more favorably (59% positive), whereas poultry PAPs (69% negative) and especially porcine PAPs (75% negative) elicited more unfavorable responses. The three RF models achieved accuracies ranging from 65% to 69% and showed that the main barriers to WTC differed by ingredient. Disgust emerged as the most influential predictor in the model for insect PAPs, while perceived product safety was the leading concern for both poultry and porcine PAPs. For these two ingredients, the item concerning the perceived naturalness of the raw material emerged as the second most important limiting factor. In terms of WTP, 60% of respondents were not willing to pay either more or less for fish fed PAPs, although the tendency leaned toward discount requests. Acceptance was highest for fish fed insect PAPs (positive WTP: 17%; negative WTP: 23%), whereas fish fed poultry or porcine PAPs were more often associated with lower WTP (positive WTP: 10%; negative WTP: 30% for both). The role of additional sustainability information was statistically significant ($p < 0.0001$), increasing the share of respondents willing to pay a premium for fish fed insect (22%), poultry (19%), and porcine (18%) PAPs, while slightly reducing negative WTP to 21%, 23%, and 26%, respectively.

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Ingredients perceived as more consistent with fish's natural diet tended to elicit more favorable reactions. FM and SBM attracted limited controversy, while PAPs, especially poultry and porcine sources, polarized respondents more strongly. Within the PAP category, insect stood out as the most accepted option. They were associated with more favorable sentiment and consistently showed the highest WTP. Proximity to fish's natural diet may foster acceptance, even though disgust remains an important barrier, consistent with previous research on indirect entomophagy (Niimi et al., 2025). By contrast, reluctance toward poultry and porcine PAPs appears to be driven primarily by perceived safety concerns, possibly reflecting the legacy of past concerns surrounding TSEs, as well as the unnaturalness of the practice. The idea emerging from the results is that insect PAPs are more accepted than the other two, possibly because they may be perceived as more natural. Although the positive effect of the information treatment indicates that targeted communication emphasizing the sustainability of PAP-based feeds could help foster consumer acceptance, future studies should focus on the role of perceived naturalness in the acceptance of these circular solutions.

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DIETARY CALCIFEDIOL SUPPLEMENTATION IN JUVENILE EUROPEAN SEA BASS (*Dicentrarchus labrax*): PRODUCTIVE, NUTRITIONAL AND HEALTH RESPONSES

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Introduction

The progressive replacement of fishmeal and fish oil by plant-based ingredients in marine fish diets has altered their micronutrient composition, increasing the need for vitamin supplementation, including vitamin D. Traditionally, cholecalciferol (vitamin D₃) has been used as the dietary source, which must undergo two sequential hydroxylations to be converted first into calcifediol and subsequently into calcitriol, its biologically active form.

Direct dietary supplementation with calcifediol (25 OHD₃) bypasses the first hepatic hydroxylation step, potentially enhancing vitamin D bioavailability and biological efficacy. In terrestrial vertebrates and some cold-water aquaculture species, such as salmonids, calcifediol supplementation has been associated with improved growth, mineral metabolism and physiological health (Rider et al., 2023; 2024; 2025). In contrast, information regarding its application in Mediterranean marine species remains scarce, with only one recent study conducted in juvenile gilthead seabream (*Sparus aurata*) (Domínguez et al., 2025). Therefore, the present study aims to provide new evidence on the effects of calcifediol supplementation in juvenile European sea bass, contributing to the nutritional knowledge base of Mediterranean aquaculture.

Materials and Methods

A total of 600 juvenile European sea bass (*D. labrax*; 62.0 ± 0.8 g) were randomly distributed among 15 tanks following a completely randomized design, with five experimental diets tested in triplicate. Diets were identical except for calcifediol supplementation levels (<2.0; 21.6; 53.5; 102.6 and 169.8 ppb). Fish were fed to apparent satiation three times daily for 113 days under a natural photoperiod.

On day 35, samples were collected for gene expression analyses, and a subgroup of fish was subjected to a *Vibrio harveyi* challenge, with survival monitored for 23 days. At the end of the trial, growth performance parameters (FCR and SGR) were calculated, and samples were collected for body composition, somatic indices, vitamin content, gene expression, enzymatic activity, digestive histology, and skeletal anomaly assessment. Data were analyzed using ANOVA and linear regression.

Survival following *Vibrio harveyi* challenge exhibited a non-linear, treatment-dependent pattern, with the highest protective effect observed at intermediate dietary calcifediol levels (21.6 and 53.5 ppb). This response was followed by a progressive reduction in survival at both lower and higher supplementation levels. Higher calcifediol concentrations (102.6 and particularly 169.8 ppb), as well as the absence of calcifediol supplementation, appeared to negatively affect the host response to infection, potentially linked to physiological stress, disruptions in homeostatic balance, or interference with immune regulatory mechanisms. Ongoing analyses of gene expression and enzymatic activity form part of the study and will support a more integrated understanding of the mechanisms underlying the responses observed during the *V. harveyi* challenge.

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Table 1. Production parameters and skeletal anomalies after 113 days of calcifediol supplementation

Analyzed caclifediol in feed (ppb)	<2.0	21.6	53.5	102.6	169.8	p-value
Final mean weight (g)	247.4±22.7	246.1±27.9	253.2±23.1	250.9±26.0	252.9±25.0	0.265
Final mean total length (g)	26.3±0.9	26.4±1.0	26.4±1.0	26.7±0.9	26.6±1.0	0.126
FCR	1.66±0.15	1.69±0.07	1.64±0.10	1.63±0.09	1.59±0.05	0.909
Skeletal anomaly frequencies (%)	3.0 ± 5.3	0.0 ± 0.0	2.6 ± 4.4	4.2 ± 5.9	13.9 ± 4.8	0.103

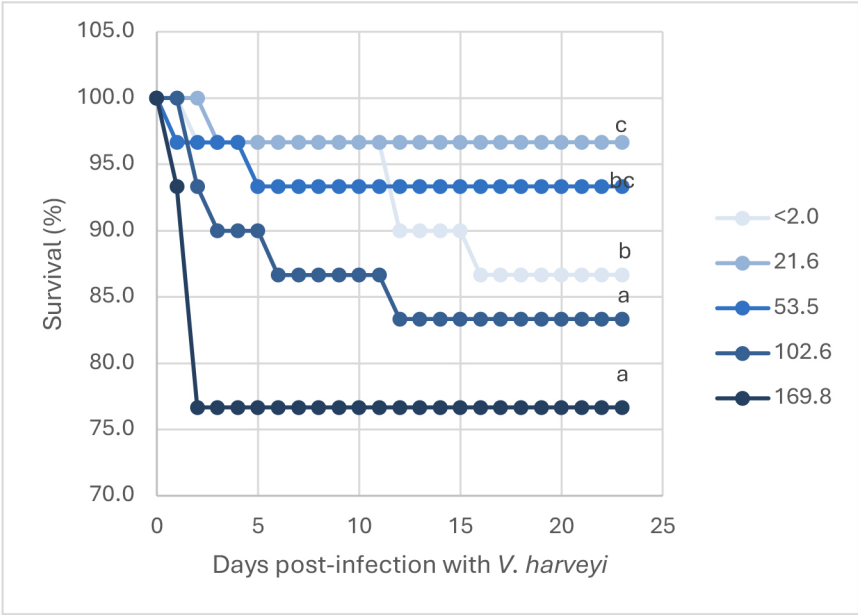


Figure 1. Survival curve after a 23-day challenge with *V. harveyi*.

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ANALYSIS OF BIOSECURITY MEASURES AND TECHNOLOGICAL LEVEL IN RAINBOW TROUT FARMS IN THE PROVINCES OF TRENTO AND UDINE (NORTH-EASTERN ITALY)

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Introduction

Italy is one of European leading producers of rainbow trout (*Oncorhynchus mykiss*), and around 70% of national production is concentrated in the north-east of the country, where the Autonomous Province of Trento (TAP) and the province of Udine are the two main production areas, due to the abundance of watercourses within their territories. The health status of a fish farm is the result of the interaction of several protective factors: among these, biosecurity and the technological level of the facility play a particularly significant role. European animal health legislation (EU regulation 2016/429, Animal Health Law) emphasizes the importance of disease prevention and identifies biosecurity as one of the main tools for reducing the risk of the introduction and spread of infectious diseases, whilst a high level of technology can promote better facility management influencing its health status. The aim of this study was to assess the biosecurity and technological level of rainbow trout farms in the provinces of Trento and Udine using a standardised methodology.

Materials and methods

The study included 57 salmonid farms, 36 of which are located in the TAP and 21 in the Province of Udine, selected from the national livestock register. The farms of interest were identified in collaboration with the local veterinary authorities (ASUIT for Trento, ASUFC for Udine) and the trade association (Italian Fish Farmers' Association (API)): specifically, all farms with the business activity 'fattening for human consumption' that kept rainbow trout, among other possible species, were selected.

Data relating to the biosecurity measures applied in each farm were collected using the checklists still in use throughout the country (Annex F of Legislative Decree 148/2008, transposing Directive 2006/88/EC). The questions of the checklists were submitted to 12 aquaculture experts with different professional backgrounds (feed industry, private practice, local competent authorities). Using the expert elicitation method, the group of experts assigned a level of importance to each biosecurity measure based on its relevance in preventing the introduction and spread of infectious fish diseases.

At the same time, each expert also assigned a score to the certainty of their answer. The weighted scores were then combined with the checklist responses collected for each farm to obtain an overall biosecurity score for the farm itself. Based on the scores obtained, the farms were divided into five categories (low, low-medium, medium, medium-high, high). Following the same logical framework, a technological level index was constructed for each farm, based on the presence or absence of specific farm technologies (which were also weighted according to importance), enabling the farms to be classified into three categories (low, medium, high).

Results

With regard to biosecurity levels, the expert elicitation analysis identified five key measures requiring particular attention: 1) Trucks' cleaning and disinfection; 2) regular removal of dead fish; 3) protection against fish-eating birds; 4) use of dedicated equipment in the various areas of the farm; 5) provision of separate areas dedicated to the loading of dead fish. By combining the weights of the measures with the data on the farms, in the TAP no farm had a low or medium-low level (0%), 31% (11 out of 36) had a medium level, 61% (22 out of 36) had a medium-high level, and the remaining 8% (3 out of 36) of farms had a high level of biosecurity. In Udine province, no farm had a low, medium-low or medium level of biosecurity (0%); 10% had a medium-high level (2 out of 21) and the remaining 90% had a high level.

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Taking the technological level into account, in Trento, only one farm was classified as low (3 %; 1 out of 36), the majority as medium (64 %; 23 out of 36) and 33 % (12 out of 36) as high. In Udine, no farm was classified as low, whilst 24 % (5 out of 21) were classified as medium and 76 % (16 out of 21) as high.

Discussion

The results show that the majority of livestock farms in the two provinces under consideration have a biosecurity level ranging from medium to high, demonstrating that the most important preventive measures are generally well implemented and that the requirements laid down in EU regulation 2016/429 and the relevant delegated and implementing acts are being complied with. The level of technology was also found to be uniformly quite good across the two provinces, demonstrating that basic technologies are now widely adopted while only a few farms use more advanced solutions. This difference between the two provinces is likely linked to the type, size and investment capacity of the farms.

Conclusions

In conclusion, the results highlight an overall good level of both biosecurity and technological advancement, although there is still scope to improve both aspects and make the sector more competitive.

Acknowledgment

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DETECTION OF TAURINE IN THE CAUDAL AND DORSAL MUSCLE OF NILE TILAPIA (*Oreochromis niloticus*) BY HPLC-UV

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Introduction

Taurine is a sulfur-containing amino acid widely recognized for its physiological functions in osmoregulation, antioxidant defense, neural development, and cardiovascular health. Due to its nutritional and functional relevance, taurine has received increasing attention in aquaculture and food science research, particularly in fish species destined for human consumption. Despite the growing interest in taurine analysis, information regarding the optimization and standardization of extraction protocols for Nile tilapia flesh by HPLC-UV without use of derivatization techniques remains limited. Therefore, this study evaluated the combined effects of muscle region, extraction solvent, and extraction temperature on taurine quantification in Nile tilapia muscle using high-performance liquid chromatography (HPLC), aiming to provide methodological support for the standardization of taurine analysis in aquaculture products.

Materials and Methods

Dorsal and caudal muscle samples from three Nile tilapia (*Oreochromis niloticus*; 588.6 ± 57.3 g) GIFT strain were frozen. To obtain the extracts, samples were thawed at room temperature; 1.0 g of each sample was homogenized with 3.0 mL water milliq using an Ultra-Turrax, incubated in a water bath at 60°C or 100°C for 60 minutes (Permata et al 2014), and centrifuged at 4000 rpm (10 min, 10°C). The supernatant was filtered through a 0.45 μ m filter and stored at 4°C for 48 hours. Additionally, in 0.1 ml from extract was add ethanol making an solution 80%, stored at 4°C for 24 hours, newly centrifuged and floating was filtered. For HPLC analysis (YL9300, YL Instrument, Anyang, Korea), a taurine standard (Sigma-Aldrich-Merck) was prepared in water at concentrations of 100, 200, 400, and 800 mmol, filtered through a 0.45 μ m filter, and 20 μ L was injected into the HPLC. A LUNA column (5 μ m, RP-18, 250 cm $\times$ 4.6 mm) was used, with separation achieved using a mobile phase consisting of 10 mM sodium acetate buffer in Milli-Q water (A) and acetonitrile (B) under the following conditions: 0–1 min (20% B); 1.01–1.5 min (5% B); 1.51–8 min (4% B); constant flow rate of 1 mL/min; detection at 254 nm; and a temperature of 35°C. Retention time and peak height were visualized using Clarity 8.1 software. The taurine standard eluted at 2.98 minutes, and a standard curve was generated, yielding the equation $y = 4.2387x - 26.406$ ($R^2 = 0.98$). The data were analyzed using a generalized linear model (GLM) assuming a Gamma distribution with a log link function. The model included the fixed effects of muscle sample, solvent, temperature and their interaction. Means were compared using Tukey's test at a 5% significance level.

Results

Taurine concentrations ranged from 1.73 ± 0.02 , to 11.71 ± 0.19 μ mol/g among the evaluated extraction conditions. Marked differences were observed according to muscle region, extraction solvent, and extraction temperature. In the caudal muscle, aqueous extraction increased taurine concentrations from 1.73 ± 0.02 μ mol/g at 60°C to 4.42 ± 0.08 μ mol/g at 100°C. Conversely, ethanolic extraction decreased taurine concentrations from 11.71 ± 0.19 μ mol/g at 60°C to 7.04 ± 0.04 μ mol/g at 100°C. The highest taurine concentration was obtained from caudal muscle extracted with ethanol at 60°C. In dorsal muscle, taurine concentrations ranged from 2.75 ± 0.33 to 3.49 ± 0.39 μ mol/g across all extraction conditions. Overall, substantial differences in taurine recovery were observed among extraction conditions. A graphical summary of taurine concentrations obtained under the different extraction conditions is presented in Figure 1.

Discussion

The results demonstrate that taurine recovery in Nile tilapia is highly dependent on extraction methodology considering the tissue characteristics. The pronounced differences observed among treatments indicate that extraction solvent, temperature, and muscle region can substantially influence measured taurine concentrations. Caudal and dorsal muscle are characterized by different fiber types, exhibits varying in lipid, aminoacids and taurine concentrations, reflecting distinct metabolic activities within the organism. The greatest variation was observed in caudal muscle, where aqueous and ethanolic extractions produced contrasting temperature-dependent responses, whereas dorsal muscle exhibited comparatively stable taurine concentrations across treatments.

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Acknowledgment

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CONSUMER ACCEPTANCE OF SUSTAINABLE AND ALTERNATIVE AQUATIC FOODS IN GREECE: PRELIMINARY RESULTS FROM THE SEAFOOD PROJECT

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Introduction

Aquatic foods provide high-quality protein, omega-3 fatty acids and essential micronutrients, contributing to improved human health and nutrition (Golden et al., 2021). In addition, aquatic foods are increasingly recognized as a more sustainable source of animal protein compared to many terrestrial alternatives and can support the transition towards environmentally sustainable food systems (Hilborn et al., 2018). Despite the strong maritime tradition of Greece, seafood consumption remains lower than in several other Mediterranean countries. Understanding consumer perceptions and willingness to adopt sustainable and alternative aquatic foods is therefore important for supporting fisheries, aquaculture diversification and sustainable dietary transitions. This study presents preliminary results from the SEAFOOD project, which aims to promote awareness and sustainable consumption of ocean-derived foods through citizen-science and educational activities.

Materials and Methods

Data were collected between April and May 2026 using a structured questionnaire developed within the SEAFOOD project. The survey included questions on demographics, seafood consumption habits, attitudes towards seafood, sustainability perceptions and willingness to consume alternative aquatic foods, including cephalopods, bivalves and marine algae. Preliminary analyses were conducted using data from 246 completed questionnaires collected in Greece. Descriptive statistics were used to characterize consumption patterns and consumer perceptions.

Results

A total of 246 questionnaires were analyzed. Most respondents reported consuming seafood regularly, with 33.7% consuming seafood once per week and 33.3% 1–3 times per month, while only 1.2% reported never consuming seafood (Figure 1A). Consumption patterns varied among seafood categories (Figure 1B). White fish (61.1%) and fatty fish (54.6%) were the most frequently consumed categories, followed by crustaceans (34.6%) and cephalopods (28.3%). In contrast, consumption of bivalves (7.6%) and algae/seaweed (4.2%) was very limited.

Respondents demonstrated positive attitudes toward sustainable seafood choices. The highest agreement was observed for preference for locally sourced seafood (79.5%) and awareness of differences in environmental footprint among seafood products (77.0%). More than half of participants agreed with considering seafood origin (56.6%), replacing commonly consumed species with lower-footprint alternatives (54.9%), and increasing the consumption of cephalopods and bivalves (53.7%). Lower agreement was reported for consuming less-known seafood species (45.5%) and acceptance of algae as part of a sustainable diet (45.1%).

Discussion

The present findings suggest that while Greek consumers currently rely predominantly on conventional fish products, there is potential for greater adoption of alternative aquatic foods that could contribute to more sustainable food systems. Despite the currently low consumption of bivalves and marine algae, respondents expressed positive attitudes toward locally sourced seafood and lower-footprint alternatives. As aquatic foods are increasingly recognized as important components of sustainable healthy diets (UN Nutrition, 2021), improving consumer familiarity with underutilized resources such as bivalves and marine algae may facilitate dietary diversification and support the transition toward more sustainable seafood consumption patterns.

Acknowledgment

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DOES DIFFERENT DIETARY FAT CONTENT AFFECT PHOSPHORUS REQUIREMENTS IN SEAWATER ATLANTIC SALMON?

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Are phosphorus requirements influenced by dietary fat content?

Phosphorus is essential for growth and bone mineralisation. The requirements for phosphorus are higher during increased growth rate. Farmed Atlantic salmon (*Salmo salar*) showed a fast growth (0.98 mm.day⁻¹) during warmer water temperatures (7-14 °C) and longer daylight between April-July compared to slower growth (0.62 mm.day⁻¹) at 5-9 °C and shorter daylight between December-April (Drábiková et al., 2026). Salmon in this study required 3.7 g/kg available phosphorus between December-April and 4.6 g/kg available phosphorus between April-July to achieve regular growth and bone mineralisation (Drábiková et al., 2026). As increased dietary fat is also linked to accelerated growth the current study assesses the dietary phosphorus requirements in seawater stages of farmed Atlantic salmon fed different fat levels.

Materials and Methods

In August 2024, salmon post-smolts (4800 fish) were pit-tagged to follow individual fish growth and deformity development. Fish were measured (fork length was 30 cm and weight 290 g on average), 2909 fish were x-rayed, and all fish were divided among 24 sea-cages (5.2 x 5.2 x 6 m, width x length x depth) located at MOWI Feed Research Station (Aukan), Averøy, Norway. Animals were exposed to natural photoperiod and temperature and fed one of 12 formulated diets. At the start of the trial additional 50 animals were sampled for base-line data.

We tested two dietary fat levels ('low' with 30 % and 'high' with 36 % fat content) and six levels of dietary phosphorus (P1-P6) with increasing monoammonium phosphate supplementation (5.4-13.2 g.kg⁻¹ total phosphorus (tP) in a randomised 2 x 6 duplicated factorial design. 'Diet P1' contained 5.4-7.2 g.kg⁻¹ tP, 'Diet P2' had 6.3-8.2 g.kg⁻¹ tP, 'Diet P3' contained 7.6-9.3 g.kg⁻¹ tP, 'Diet P4' contained 9.2-12.0 g.kg⁻¹ tP, 'Diet P5' was formulated according to currently used levels in commercial diets for different salmon stages and contained 8.2-10.4 g.kg⁻¹ tP, and 'Diet P6' contained 10.3-13.2 g.kg⁻¹ tP.

Following the start of the trial, salmon were individually measured in December, May, and August with 20, 12, and 33 fish sampled per cage at the respective sampling points. For the purpose of this abstract we will discuss results from the final sampling done in August 2025. The analysis included vertebral deformity assessment using x-ray images, total mineral content (ash) of vertebrae as representatives of endoskeleton, total ash content of scales as representatives of dermal skeleton, and scale mineralisation using whole mount Alizarin red stain. Alizarin red binds to calcium, i.e. mineralised part of the scale, while the non-mineralised part remains transparent but visible under brightfield light with partly closed aperture diaphragm. Assessment of scales represents a valuable non-invasive tool to assess severity of phosphorus deficiency in fish.

High energy increased salmon growth when combined with 'Diet P2'

Interestingly, at the final sampling in August, salmon fed 'Diet P2 high fat' were significantly longer (67.7 cm on average) and heavier (4200 g on average) compared to salmon fed 'Diet P2 low fat' with an average fork length of 64.9 cm and weight of 3868 g. As a result only 'Diet P2 low fat' fed salmon were significantly shorter and lighter than fish fed 'Diet P3-P4' at both fat levels with an average fork length and weight of 67.8 cm and 4200 g. Irrespective of dietary fat content, Atlantic salmon fed 'Diet P1' showed significantly reduced fork length and weight (58.7 cm, 3046 g for 'low' fat and 59.6 cm, 3020 g for 'high' fat diet groups) compared to salmon fed 'Diets P2-P6' at both fat levels.

Scale mineralisation was challenged into a greater extent than vertebrae mineralisation

At final sampling in August scale ash of salmon fed 'Diets P1-P2' at both fat levels as well as salmon fed 'Diet P3' at low fat level was significantly reduced compared to the remaining diet groups (Figure 1a). In contrast, vertebrae ash was significantly reduced only in salmon fed 'Diets P1-P2' at both fat levels relative to salmon fed 'Diets P4-P6'. Vertebrae ash of salmon fed 'Diet P3' was comparable to 'Diets P4-P6' and to those fed 'Diet P2' at high fat level (Figure 1b). Uniquely, scale mineralisation observed and quantified on whole mount Alizarin red stained scales was reduced not only in salmon fed 'Diet P1' but also in salmon fed 'Diet P2' with low fat content.

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What does this mean for skeletal health?

The observed reduction in growth and skeletal mineralisation will be elaborated in respect to the vertebral column health while potential to compensate phosphorus deficiency through the resorption of scales will be considered. We will discuss the possible advantages of either low or high fat levels with varying dietary phosphorus levels for phosphorus digestibility and reduced phosphorus excretion in seawater stages of Atlantic salmon and discuss the effect on dietary phosphorus requirements.

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BIOCHEMICAL FINGERPRINTING OF PARASITIC SEA LICE (*Lepeophtheirus salmonis*) USING MID-INFRARED SPECTROSCOPY AND STABLE ISOTOPE ANALYSIS FOR HOST ORIGIN ASSIGNMENT

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Background

The salmon louse, *Lepeophtheirus salmonis*, is associated with substantial losses to the salmon farming industry. These ectoparasites feed on the blood, gills, and skin of their hosts, facilitating pathogen entry and reducing immune function, leading to secondary infections. Adverse health outcomes and mass mortalities are increasing alongside the emergence of treatment resistance; therefore, the sustainability of salmon aquaculture depends on a shift towards prevention-focused sea lice control. At present, a limited understanding of sea lice transmission dynamics represents a major barrier to effective prevention. Due to the highly dispersive nature of free-living larvae and migratory salmonids, sea lice from distinct sources exhibit weak genetic differentiation (Jacobs et al., 2018)2018. Accurate determination of host origin at individual louse level would substantially advance our understanding of sea lice transmission dynamics, relationship between farmed and wild salmonid host populations, and provide evidence base for more targeted and effective management interventions.

Alternative profiling approaches to traditional genetics, such as stable isotope analysis (SIA) and mid-infrared reflectance spectroscopy (MIR), remain underexplored for assigning the parasite origin of individual sea lice. Parasite isotopic signatures reflect host diet; initial studies suggest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values can distinguish farmed from wild lice due to contrasting feed sources (Butterworth et al., 2004). However, SIA analysis is costly, and studies in other organisms suggest infrared spectroscopy may provide a cost-effective proxy (Sim et al., 2023). MIR can also detect differences in parasite tissue composition associated with variation in dietary lipid and carbohydrate content. This pilot study used adult sea lice from farmed salmonid hosts (n=35) and wild salmonid hosts (n=106) in Loch Etive, Scotland, to compare the discriminatory power of SIA and MIR in determining sea lice host origin. All samples underwent carbon and nitrogen SIA and MIR spectroscopy, and a subset (n=40) also underwent low-coverage whole genome sequencing, and mitochondrial haplotype frequencies were compared to provide a genetic benchmark.

Results and Discussion

Wild- and farmed-host derived sea lice shared the same fundamental spectral peaks, reflecting identical biochemical classes, but differed consistently in absorbance intensity (figure 1). MIR spectroscopy discriminated sea lice from farmed and wild hosts using partial least square discriminant analysis with a balanced accuracy of 0.838 under leave-one-out cross-validation, confirmed as non-random by permutation testing ($p < 0.001$). The primary discriminating signal localised to the CH_2/CH_3 bending region ($1350\text{--}1480\text{ cm}^{-1}$; Cohen's $d = 1.311$), with lice from farmed hosts exhibiting consistently higher aliphatic lipid absorbance across 100% of wavenumbers in this region after FDR correction. Supporting differences were observed in the amide II ($1480\text{--}1580\text{ cm}^{-1}$; Cohen's $d = 1.024$) and phosphodiester/amide III ($1200\text{--}1280\text{ cm}^{-1}$; Cohen's $d = 0.719$) sub-regions at 63% and 56% significance, respectively. In contrast, sea lice from wild hosts showed higher absorbance in the O–H/N–H stretching ($3200\text{--}3600\text{ cm}^{-1}$) and low-wavenumber ($400\text{--}600\text{ cm}^{-1}$) regions, consistent with greater hydration and variability in their biochemical composition. Mean isotope values differed between groups, with $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, and $\delta^{34}\text{S}$ in farmed-origin lice depleted than in wild-origin lice by $3.92 \pm 0.15\text{‰}$, $4.34 \pm 0.18\text{‰}$, and $2.96 \pm 0.16\text{‰}$, respectively.

These differences are consistent with expectations of a more uniform and lipid-rich diet in farmed salmonid hosts and greater environmental variability in wild salmonids. Together, this work provides proof of concept for using biochemical markers to trace sea lice origin, supporting improved understanding of transmission pathways and more targeted strategies to improve salmon health.

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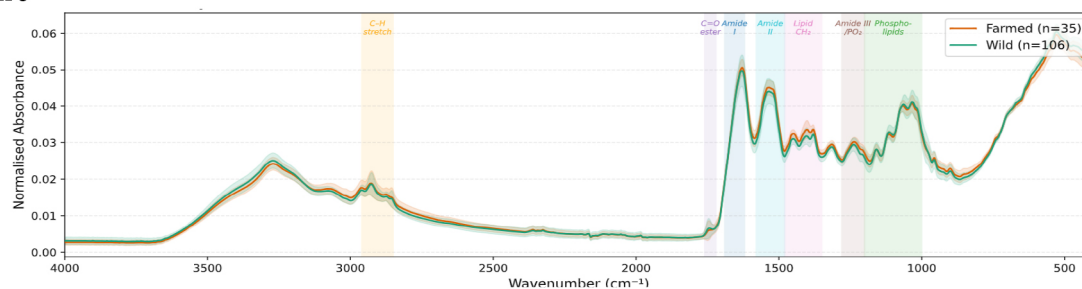
Figure

Figure 1. Biochemical interpretation of spectral differences between farmed and wild sea lice (*Lepeophtheirus salmonis*).

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INTEGRATED MULTITROPHIC AQUACULTURE OF MULLET, CLAM, OYSTER AND ALGAE IN COASTAL PONDS ON LAND AFFECTED BY SALINISATION IN THE EBRO DELTA, SPAIN

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Introduction

Integrated multi-trophic aquaculture (IMTA) is a sustainable production system that promotes nutrient recycling to minimise the accumulation and discharge of waste (Chopin et al., 2012). BLUEBOOST aims to promote the adoption of technologies based on the co-cultivation of low-trophic-level species with established fed species by providing knowledge on IMTA culture technologies and the systems environmental and economic benefits. In addition, IMTA offer potential for seafood production and ecosystem regeneration in environments that are under threat from climate change and anthropogenic pressures. The Ebro Delta, in common with many sea-level coastal areas, is under threat from climate change and land has become salinised due to reduced sediment discharge from the river, sea-level rise and storm intensification. Although IMTA systems have great potential for more sustainable aquaculture and ecosystem restoration, their application by the aquaculture sector remains limited by technical complexity and the scarcity of data to demonstrate the environmental and economic benefits (Ghosh et al., 2025). This work presents the results of a trial developed in Spain, in coastal ponds of the Ebro Delta. The experiment aims to compare a monoculture system with an IMTA system that can be positioned on salinised land to provide protection from climate change-influenced coastal processes.

Materials and Methods

The study was conducted in IRTA, La Ràpita Centre (Catalonia, Spain), between November 14, 2024, and October 20, 2025. Three coastal ponds of 100 m³ and 1 m depth, located in the Ebro Delta, were used. Seawater was pumped from the bay into a covered reservoir and pumped to the ponds without prior treatment. A venturi on the water inlet pipe maintained high oxygen levels close to the inlet point. A single monoculture pond was compared to two IMTA ponds. Each pond was stocked with 437 flathead grey mullet (*Mugil cephalus*) (53.9 ± 32.0 g weight) that were obtained from IRTA's own production. Each IMTA pond was also stocked with 1000 European flat oyster (*Ostrea edulis*) (15.1 ± 2.5 mm) obtained from The Oyster Restoration Company (TORC, Scotland), 1000 Pacific oyster (*Magallana gigas*) (15.4 ± 2.3 mm) obtained from Musclarium (Catalonia, Spain), 3161 Manila clam (*Ruditapes philippinarum*) (18.7 ± 1.1 mm) obtained from Proameixa Acuicultura (Galicia, Spain), and Ulva sp. (25 kg) from IRTA's own production. The mullet were fed ad libitum with a commercial feed (DIBAQ CATFISH 42, 4.0 mm). Daily mortalities were recorded, and biometric sampling was conducted every three months. Samples were also taken for nitrogen, phosphorus, and carbon analysis. Temperature was measured with sensors in the water column and sediment (HOBO, USA). Dissolved oxygen was measured twice daily, and pH and salinity once daily using a ProQuatro multiparameter meter (YSI, OH, USA). Inflow and outflow water samples were taken twice weekly to determine *in vivo* chlorophyll by fluorimetry, and every two weeks for nutrient analysis.

Results and Discussion

During the trial, environmental conditions ranged from 9 to 31 °C, dissolved oxygen from 2 to 10 mg/L, pH from 5.80 to 9, salinity from 26 to 38 ppt, and *in vivo* chlorophyll levels from 0.3 to 8 mg/L in the inlet water and from 0.3 to 14.3 mg/L in the outlet water. On October 12, 2025, a torrential rainfall event exceeding 300 L/m² occurred, causing a sharp decrease in salinity and significantly impacting the final survival of the bivalves. Mullet reached final weights of 342.25 ± 116.20 g and 354.83 ± 165.23 g in the IMTA ponds, and 401.30 ± 148.19 g in the monoculture. Although growth was slightly higher in monoculture, survival was greater in IMTA (65.8% and 69.0%) than in monoculture (50.9%). The proportion of survivors showed significant differences between each IMTA pond and the monoculture (chi-square test; $p < 0.0001$ in both cases), with no differences between the two IMTA ponds. The greater final weight in monoculture could be related to a lower final density due to mortality, while all three ponds reached a similar final biomass. The Pacific oyster reached 52.94 ± 12.82 mm and 47.83 ± 8.86 mm in length, with survival rates of 86.1% and 92.1% until August, respectively, but final mortality was 100% after the rain event. The European flat oyster showed slower growth (34.15 ± 8.94 mm and 26.17 ± 9.33 mm) and

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low final survival (2.3% and 2.1%) with mortalities associated with high summer temperatures. The Manila clam reached a large market size of 35.55 ± 2.22 mm and 33.93 ± 1.67 mm. Clam survival of 80% and 92% were recorded in August. However, the final survival rate was 22.2% and 33.5%. During final sampling, there was no difference in the average shell size between live and dead clams, suggesting that much of the mortality occurred shortly before the final sampling (20 Oct.) that followed the torrential rain event (12 Oct.). These results point to the clam as the most promising species in the system with growth to harvest size in a short growing period and potentially high survival. Mullet also grew well and demonstrated robustness to extreme changes in the environment, both salinity and temperature. *Ulva* sp. showed increases of 53% and 281% in a six-week cycle, although optimisation of its support and harvesting will be necessary. Overall, the results support the potential of the evaluated IMTA system. The species appear to interact positively, which needs to be confirmed by analysis of nutrient flows through the system. The system can also give economic use to abandoned salinised land to support ecosystem restoration.

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HYPERSPECTRAL CHARACTERIZATION OF PHYSIOLOGICAL RESPONSES IN *Palmaria palmata* TO OPTIMIZE LAND-BASED ENVIRONMENTAL PARAMETERS

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Background:

Seaweed farming is one of the fastest-growing sectors of European aquaculture. Despite the current market dominance of brown seaweed, native red seaweeds like dulse (*Palmaria palmata*) offer superior nutritional and phytochemical profiles, driving increased commercial interest. However, cultivation practices for these species remain in their infancy, with no established consensus on the environmental conditions required to optimize growth. Furthermore, while the antioxidant and phytochemical content of red seaweeds is a primary asset, quantifying these compounds currently requires labor-intensive laboratory procedures. In this study, we evaluated the effects of light spectra, simulated at depths of 0.5, 5, 10, and 20 meters, on the growth and physiological health of *P. palmata* within land-based systems. Using hyperspectral imaging, we characterized biochemical shifts in tissue composition across these light environments. By pairing spectral data with empirical phytochemical concentrations measured via mass spectrometry, we developed statistical models to assess the accuracy of rapid imaging as a high-throughput proxy for chemical quantification.

Results:

Preliminary results indicate that dulse populations from the Swedish west coast exhibit significantly higher growth rates under light conditions simulated at ~5m depth. Light spectra also influenced chemical composition; macroalgae grown at the shallowest simulated depth (0.5m) displayed spectral profiles distinct from those cultivated under deeper-water light conditions (Figure 1). Furthermore, we observed significant intra-thallus spectral variation along the length of the fronds. These gradients transitioned from the older, thicker base tissue to the newly developed tips, likely reflecting the maturation of photosystems as tissue ages. The extent of this transition zone (tip vs base) varied between treatments, likely reflecting not only the differential growth rates we observed, but also the quality of available light. This physiological shift is critical for cultivation strategy: while younger tissue drives biomass accumulation, older tissue may sequester higher concentrations of high-value carotenoids. Consequently, optimizing culture conditions solely for longitudinal growth may inadvertently produce less robust algae with diminished concentrations of the compounds that drive their market value.

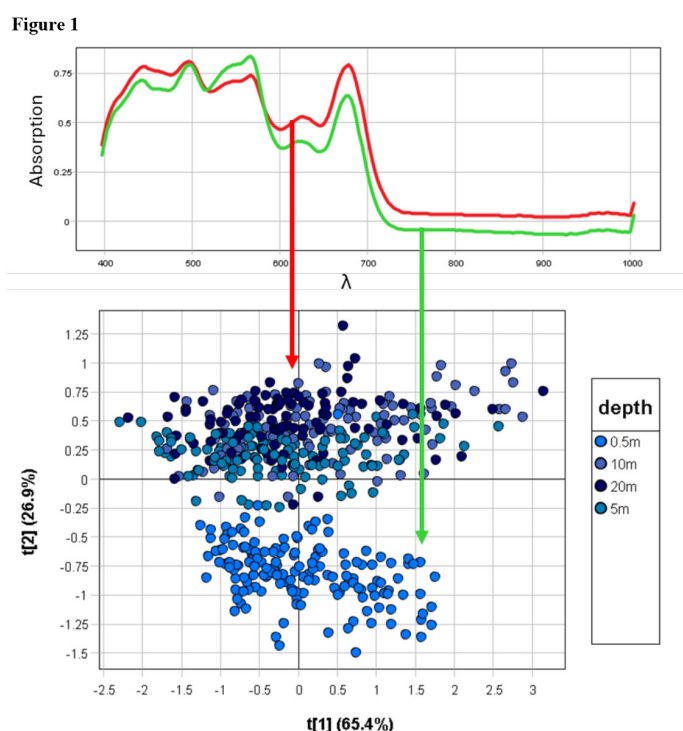


Figure 1: TOP: Spectral profile of dulse grown at 0.5m and 20m simulated light conditions (green and red, respectively). BOTTOM: PCA of all individuals, based on average spectral profile.

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Acknowledgment

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EFFECT OF A LIGNOSULFONIC ACID-BASED ACIDIFIER ON VIBRIO CONTROL AND PRODUCTION IN *Penaeus vannamei*

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Introduction

Vibrio spp. are among the most significant bacterial pathogens in shrimp aquaculture, contributing to high mortality and reduced production efficiency worldwide. Vibriosis remains a major disease affecting *Penaeus vannamei*, with species such as *Vibrio harveyi* and *Vibrio parahaemolyticus* frequently associated with outbreaks (Bhassu et al., 2024). The increasing prevalence of antimicrobial resistance in *Vibrio* populations further complicates disease control strategies (Hobe et al., 2026).

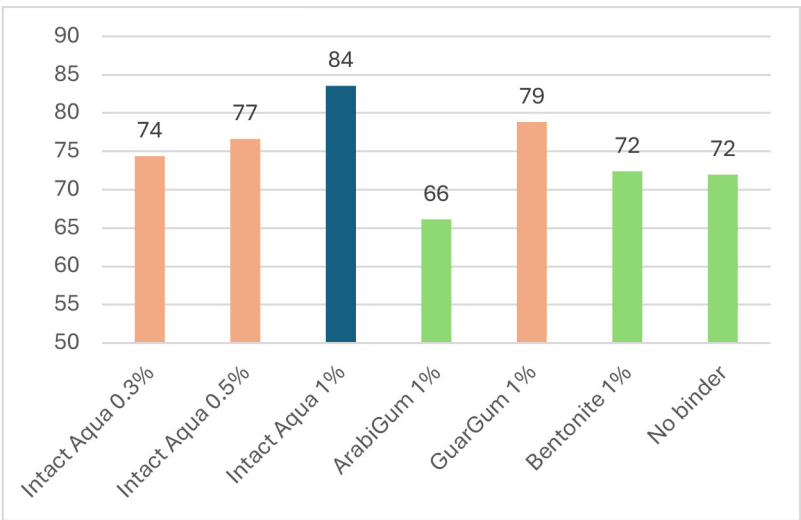
Virulence in these bacteria is closely linked to quorum sensing (QS), a regulatory system controlling gene expression, biofilm formation and pathogenicity (Ye et al., 2025). Disruption of QS (quorum quenching) is increasingly recognized as a promising and sustainable approach to control bacterial diseases in aquaculture (Saranya et al., 2025).

This study combined in vitro and in vivo approaches to evaluate the effect of a lignosulfonic acid-based feed acidifier on *Vibrio* control and shrimp performance.

Materials and Methods

In vitro assays were conducted to assess quorum sensing (QS) inhibition and biofilm formation in pathogenic bacteria (*Vibrio harveyi*, *Vibrio campbelli*, *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Pseudomonas* sp.). These analyses were performed at an accredited microbiological laboratory in Ecuador, where bacterial luminescence and biofilm formation were quantified using microplate assays following standard optical density methods. Bacteria were exposed to increasing concentrations (1000–2500 ppm) of a lignosulfonic acid-based acidifier.

In parallel, a 42-day feeding trial was conducted at the Laboratory of Aquatic Animal Nutrition (LABOMAR), Institute of Marine Sciences, Federal University of Ceará (Brazil). Juvenile *Penaeus vannamei* were reared under intensive outdoor tank conditions (75 shrimp m⁻²) and fed diets supplemented with 0, 5 and 15 g kg⁻¹ of the acidifier. Growth performance, survival and biomass production were monitored throughout the experimental period.



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Results and Discussion

The lignosulfonic acid-based acidifier demonstrated strong inhibitory effects on quorum sensing (QS) activity. Complete suppression of luminescence (100%) was observed for *Vibrio harveyi* and *Vibrio campbelli* at concentrations as low as 1000 ppm, indicating effective disruption of bacterial communication mechanisms. In contrast, a reference acidifier required higher concentrations (1500–1900 ppm) to achieve similar inhibition, highlighting the higher efficacy of the tested formulation.

Biofilm formation was also significantly affected. The acidifier achieved complete inhibition (100%) of biofilm formation in *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio campbelli* at 1200 ppm, while full inhibition for *Vibrio harveyi* and *Pseudomonas* sp. was observed at 1600 ppm. At lower concentrations (1000 ppm), partial inhibition was already evident, with biofilm formation reduced to 12.3% in *Vibrio parahaemolyticus* and 4.2% in *Vibrio harveyi*. These results indicate a dose-dependent response and a strong capacity to prevent bacterial adhesion and colonization.

In the feeding trial, shrimp survival increased markedly from 55.8% in the control group to 68.0% and 99.9% in the 5 and 15 g kg⁻¹ treatments, respectively. This resulted in a substantial increase in biomass production, with the highest inclusion level yielding more than a tenfold increase compared to the control. Although individual growth rates were slightly lower at higher inclusion levels, this was primarily associated with differences in survival and stocking density dynamics rather than reduced feed efficiency.

The consistency between in vitro antimicrobial activity and in vivo performance suggests that the acidifier reduces pathogenic pressure in the production system. The combined inhibition of quorum sensing and biofilm formation likely limits *Vibrio* colonization and virulence, contributing to improved survival and overall production outcomes.

Conclusion

A lignosulfonic acid-based acidifier demonstrated strong inhibitory effects on quorum sensing and biofilm formation of pathogenic bacteria, which translated into improved survival and production performance in *Penaeus vannamei*. These findings support the use of quorum sensing-targeted strategies as effective and sustainable tools for managing *Vibrio*-related challenges in shrimp aquaculture.

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THE IMPACT OF VARIOUS COPPER NANOPARTICLES AND THE IONIC COPPER ON RAINBOW TROUT (*Oncorhynchus mykiss* W.) SPERMATOZOA MOTILITY

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The amount of the nanoproducts released into the environment is a growing threat to organisms. There is little data on the effects of nanoproducts made of different compounds of the same element on biological objects. The copper nanoproducts are widely utilized due to their antimicrobial properties in feed industry, cosmetics, medical drug designing, and numerous others applications. Spermatozoa as a cell is a good model for studying the ecotoxic effects of nanoproducts and other chemicals on cells. The direct effect of two copper nanoproducts of Cu NPs and CuO NPs and copper ions (CuSO₄) on spermatozoa motility was previous investigated by our group by adding pollutants to the environment activating the gametes (Kowalska-Górska et al., 2019). More harmful effect of ionic form than nanoparticles was obtained in the study while diverse effects of the nanoproducts was not clear. The present study aimed to analyse the effect of these two copper nanoparticles and metal ions on rainbow trout spermatozoa during incubation, following prolonged exposure.

Nanoproducts of Cu and CuO (Cu NPs, CuO NPs) of primary particle size around 50 nm and ionic solution of CuSO₄ were used for the study. Suspension of concentration 0, 1, 5, 10, 25 and 50 mg Cu L⁻¹ as Cu NPs, CuO NPs and CuSO₄ was dissolved in an artificial seminal plasma. Milt was mixed with prepared solution and stored in a fridge for 96 h. At the defined incubation time, spermatozoa were activated for movement. Six motility parameters were evaluated using an automated system (CASA).

Copper nanoproducts made from different compounds have a different effect on cells. Cu NPs was more harmful than CuO NPs. The effect of Cu NPs was similar to an ionic form of CuSO₄. When incubated, the copper nanoproducts and ionic form exert a slightly positive effect on spermatozoa velocity, linearity, and motility duration, particularly in the initial hours of storage.

Funding

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BEYOND BIGGER SMOLTS: WHY PHOTOPERIOD BALANCE MATTERS FOR DEVELOPMENT IN ATLANTIC SALMON

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Smoltification in Atlantic salmon is often operationally assessed through growth, size, and seawater tolerance. However, the biological smolt is produced by a tightly timed developmental program in which winter photoperiod history, increasing spring daylength, brain remodeling, endocrine activation, and osmoregulatory remodeling across the gill, intestine and kidney are coordinated. Evidence from neuroanatomical, endocrine, molecular, and physiological studies shows that continuous constant light can uncouple this program: fish may grow well, but key smolt-related changes in retinal–preoptic connectivity, CRF neurogenesis, thyroid hormone, growth hormone, cortisol signaling, together with coordinated development of NKA enzyme activity across the three osmoregulatory organs and subsequent seawater performance may be blunted or incomplete.

This presentation revisits the concept of smolt quality from a developmental perspective. We argue that smoltification is not simply growth plus seawater tolerance, but a seasonal brain-endocrine-osmoregulatory remodeling process shaped by photoperiod history. Thus, the use of continuous light (24:0) without a preceding winter signal may produce “pseudo-smolts” that acquire some smolt characteristics, while key aspects of developmental timing, endocrine activation and osmoregulatory remodeling may remain partly or completely uncoupled, with potential consequences for long-term seawater performance. Such mismatches between photoperiodic signaling and physiological development may also contribute to production disorders including nephrocalcinosis, looser syndrome (stunts), hemorrhagic smolt syndrome (HSS), and broader smolt quality problems. These disorders may therefore, at least partly, reflect mismatches between the biological requirements of salmon and the environmental conditions provided during intensive land-based production. The objective is to remind producers, researchers, and technology providers that photoperiod balance remains a core welfare and performance factor in modern smolt production, while its wider physiological consequences are still not fully understood. Optimized lighting protocols should preserve the biological logic of winter preparation and spring progression, rather than treating continuous light as a simple growth accelerator, as they may contribute to several production disorders currently observed in land-based salmon aquaculture

Acknowledgment

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DIFFERENCES IN THE EXPRESSION OF IMMUNE-RELATED GENES IN RAINBOW TROUT (*Oncorhynchus Mykiss*) INTESTINAL CELLS ISOLATED *ex vivo* FOLLOWING EXPOSURE TO VARIOUS YEAST CELL WALL PRODUCTS

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Introduction

Infectious diseases represent a major constraint on sustainable aquaculture production, driving substantial economic losses and raising significant animal welfare concerns (Hadiuzzaman et al., 2022; Vallejos-Vidal et al., 2021). In the absence of broad-spectrum prophylactic measures, the aquaculture sector has increasingly adopted non-antibiotic immunostimulants to enhance host immune competence (Vetvicka & Vetvickova, 2020). Among these, β -1,3/1,6-glucans derived from yeast cell walls (YCW) have attracted particular interest due to their capacity to act as pathogen-associated molecular patterns (PAMPs) and microbial-associated molecular patterns (MAMPs), thereby activating innate immune signalling pathways in both freshwater and marine teleost species (Rodrigues et al., 2020; Machuca et al., 2022). Upon recognition by pattern recognition receptors (PRRs) — including functional homologs of C-type lectin receptors — β -glucans trigger downstream activation of nuclear factor- κ B (NF- κ B) and the transcriptional upregulation of pro-inflammatory cytokines such as interleukin-1 β (IL-1 β) and tumour necrosis factor- α (TNF- α) (Rodrigues et al., 2020; Hadiuzzaman et al., 2022). Despite broad evidence of their immunostimulatory efficacy, comparisons between YCW products differing in yeast origin remain limited, and the influence of production method on the quality of the immune response has not been thoroughly characterised.

Materials and Methods

Immune modulatory effects were assessed using the rainbow trout (*Oncorhynchus mykiss*) intestinal epithelial cell line RTgutGC (passage 81), derived from a distal intestinal segment of an adult female and provided by aQuaTox-Solutions GmbH (Winterthur, Switzerland). Cells were exposed to one of four treatments: (1) exposure medium L-15/ex only (negative control; Neg. CON); (2) lipopolysaccharide (LPS) from *Escherichia coli* (positive control; Pos. CON); (3) a primary-grown yeast cell wall product (Primary-YCW; TechnoMos®, Biochem GmbH, Lohne, Germany); or (4) a by-product ethanol yeast cell wall preparation (Ethanol-YCW). All treatments were applied at equivalent concentrations under standardised exposure conditions. Following incubation, total RNA was extracted and reverse-transcribed, and relative gene expression of three immune markers — IL-1 β , TNF- α , and the Toll-like receptor 4 (TLR4) interactor with leucine-rich repeats (TRIL) — was quantified by quantitative polymerase chain reaction (qPCR). Expression data were normalised to appropriate reference genes and analysed for statistically significant differences between treatment groups ($P < 0.05$).

Results and Discussion

Both YCW products elicited statistically significant upregulation of IL-1 β and TNF- α relative to the negative control ($P < 0.05$), demonstrating immunostimulatory activity at the intestinal epithelial level. Critically, gene expression levels for both cytokines remained substantially lower than those induced by LPS, indicating that neither product provoked a hyper-inflammatory response. This profile of calibrated immune activation — sufficient to prime innate defence mechanisms without triggering deleterious inflammation — is considered functionally desirable in aquaculture settings, where excessive immune activation incurs energetic costs and compromises productive performance (Hadiuzzaman et al., 2022; Vetvicka & Vetvickova, 2020).

A pronounced differential response was observed for TRIL, a TLR4 accessory molecule previously identified in teleost fish and implicated in the modulation of both LPS and viral RNA recognition (Pietretti et al., 2013). The Primary-YCW induced TRIL expression at a level statistically equivalent to LPS stimulation, whereas Ethanol-YCW produced only a modest, albeit significant, upregulation above the negative control. Because elevated TRIL expression enhances PRR sensitivity to circulating PAMPs, a higher level of TRIL induction may confer an earlier and more rapid pathogen detection capacity — an effect analogous to a broad-spectrum, unspecific innate immune priming (Pietretti et al., 2013). Importantly, this TRIL-mediated sensitisation occurred without corresponding amplification of pro-inflammatory cytokine transcription, suggesting that the tested primary-grown YCW decouples heightened pathogen surveillance from overt inflammatory cascades.

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These findings are consistent with and extend prior reports demonstrating that the immunostimulatory potency of YCW products is contingent on yeast origin and production method (Machuca et al., 2022; Rodrigues et al., 2020). Published data on the pathogen-binding capacity of the mannan oligosaccharide (MOS) fraction further indicate that primary-grown yeast-derived MOS binds approximately 100 % more pathogens than MOS derived from ethanol yeast (Eckart, 2024), corroborating the functional superiority of primary-grown YCW products across multiple immune-relevant parameters.

Acknowledgement

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EFFECTS OF FISH CAGE–CULTURE ON ZOOPLANKTON ABUNDANCE IN THE TROPICAL RESERVOIR OF ITAPAJI, SOUTH-WEST, NIGERIA

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Introduction

Zooplankton are important components in the aquatic food web, serving as a vital link between primary producers and the higher trophic level, their abundance is dictated by prevailing environmental conditions, making them a key indicator of environmental change. Over the years, there have been growing concerns about the impact of cage culture on plankton communities, associated with the increasing adaptation of cage aquaculture. The limited information on the effects of cage culture on zooplankton population in Itapaji geared this study. The study assessed the impact of fish cage culture on zooplankton abundance and water quality in Itapaji Reservoir, southwestern Nigeria.

Materials and Methods

Four cages located 50m equidistantly and tagged A, B, C, and D with cage D located upstream. Water quality and zooplankton abundance were investigated before stocking (BS), during culture (DC), before harvesting (BH). Water samples for zooplankton identification were collected from the points using a Juday net as described by Vinogradov et al., (1989), individual species were then examined under an inverted microscope at a magnification of x40 and x100 as described by Harris et al., (2000) while counting and taxonomic identification were done using a Sedgewick Rafter Cell under an inverted electron microscope (Conway et al., 2003; Kyewalyanga & Malesa 2024).

Water samples collected per point and period were measured for Dissolved Oxygen (DO), pH, temperature, phosphate, and nitrate. DO and pH were measured in situ using a CTD multi-probe (Sea-Bird Electronics, Model 19-03), and phosphate was measured in the laboratory as described by APHA (1998). Analyses were carried out using ANOVA to access spatial and temporal variation, the result expressed as mean $\pm$ standard error.

Results and Discussion

Zooplankton species identified taxonomically using appropriate keys were a total of 13,912 individuals (BS = 49.7% [6914 individuals], DC = 26.9% [3737 individuals] BH =23.4% [3261]) belonging to 3 taxonomic groups (protozoan = 70.9% [9869 individuals], rotifers=15.2% [2116 individuals] and crustacea = 13.9% [1927 individuals]) with 9 species (Euglena[10.8%], Chilomonas [0.9%], Frontonia [21.1%], Coleps [5.3%], Rotaria [15.21%], Cypridopsis[13.9%], Vorticella [3.9%], Paramecium [25.2%], and Chilodonella [3.7%]). Spatial variations in zooplankton abundance between cage sites were observed from the Principal Component Analysis (PCA), BS had PC1 (76.4%) and PC 2 (20.3%), DC had PC 1 (70.0%), PC 2 (19.8%)and PC 3(10.2%) and BH had PC1 (67.3%) and PC 2 (27.8%) accounting for the total proportion of zooplankton species.

The mean values of water quality parameters measured were dissolved oxygen (BS= 4.73 ± 0.34 mg/L, DC = 3.72 ± 0.13 mg/L, BH = 3.40 ± 0.11 mg/L), temperature (BS= 28.45 ± 0.10 oC, DC = 27.30 ± 0.13 oC, BH = 27.09 ± 0.10 oC), pH (BS= 6.19 ± 0.12 , DC = 6.05 ± 0.10 , BH = 5.73 ± 0.10), phosphate (BS= 0.21 ± 0.01 mg/L, DC = 0.17 ± 0.02 mg/L, BH = 0.12 ± 0.01 mg/L), and nitrate (BS= $0.001.19 \pm 0.00$ mg/L, DC = 0.001 ± 0.00 mg/L, BH = $0.001.00 \pm 0.00$ mg/L). The result reveals that dissolved oxygen and phosphate levels decreased during culture (DC) and before harvesting (BH), although all measured water quality parameters remained within the recommended limits for aquatic life ($p > 0.05$).

This study revealed that the zooplankton abundance varied due to the prevailing cage activities, and it is therefore essential to maintain a healthy and environmentally sustainable fish cage culture practice for the sustenance of aquatic biota.

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Acknowledgements

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EXPERIMENTAL CHALLENGE MODELS FOR *Ecytonucleospora hepatopenaei* IN SHRIMP: A COMPREHENSIVE REVIEW OF EXPERIMENTAL APPROACHES AND KNOWLEDGE GAPS

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Ecytonucleospora hepatopenaei (EHP) is a microsporidian parasite that causes hepatopancreatic microsporidiosis in shrimp, leading to growth retardation and significant economic losses in global shrimp aquaculture. Although EHP does not typically cause high mortality, its substantial economic impact due to severe growth retardation and subclinical infection makes experimental challenge models essential for studying infection dynamics, host pathogen interactions, and mitigation strategies. However, existing EHP challenge models vary widely in methodology, reproducibility, and infection outcomes. This review critically evaluates current experimental challenge models, highlighting methodological differences, advantages, limitations, and critical knowledge gaps to improve standardization and reproducibility. A structured literature review was conducted using major scientific databases to identify studies on EHP challenge models in shrimp and synthesize current methodologies.

EHP challenge models were categorized into five groups: indirect transmission (cohabitation), enteric exposure models, environmental exposure models, artificial systemic models, and alternative hosts and vectors. Cohabitation models represent biologically relevant approaches for natural transmission between infected and naïve shrimp, although they often introduce variability in infection timing and intensity. In most cohabitation trials, infection was typically detected between 10 and 21 days post inoculation (dpi), with prevalence increasing progressively over time (Tang et al., 2016; Salachan et al., 2017). However, cohabitation models often showed greater variability compared to direct inoculation methods due to limited control over inoculum dose and environmental factors.

Enteric exposure models, including feeding of infected hepatopancreatic (HP) tissue, fecal matter, oral gavage, and reverse gavage, provide biologically relevant routes of infection but are influenced by inoculum quality and shrimp feeding behaviour. Feeding infected HP tissue is one of the most commonly used methods, due to its simplicity and biological relevance with infection typically detected within 7-14 dpi (Salachan et al., 2017; Mai et al., 2020). Fecal string based inoculation (Aranguren Caro et al., 2021, 2023) and oral gavage using purified spores (Munkongwongsiri, 2021) approaches demonstrated dose-dependent infection dynamics, although infection efficiency varied across studies. Reverse gavage studies reported higher infection consistency and sustained pathogen proliferation (Mai et al., 2020).

Environmental exposure models, including immersion and waterborne transmission (Pattarayingsakul et al., 2022), provide non-invasive approaches but often result in lower infection consistency and delayed infection onset. Artificial systemic models, such as injection into hepatopancreas, ventral sinus, or muscle, although less biologically relevant, offer controlled experimental conditions for mechanistic studies (Mai et al., 2020). In addition, several studies have explored alternative hosts and potential vectors, including polychaetes, *Artemia*, and other aquatic organisms, as sources of infection or transmission pathways. However, evidence for sustained replication in these organisms remains limited, and their role in challenge model development requires further validation.

Across studies, infection onset ranged from 5-30 days post challenge, and varied widely depending on inoculation method, inoculum quality, and shrimp developmental stage. Quantitative infection assessment using qPCR is inconsistently applied, and environmental parameters are insufficiently reported. These inconsistencies contribute to inter-study variability and limit reproducibility. Standardization of inoculum preparation, dose quantification, and reporting of experimental conditions is therefore essential to improve challenge model reliability and reproducibility.

This review highlights the urgent need for standardized EHP challenge protocols and proposes key methodological considerations to improve reliability, reproducibility, and comparability of future experimental studies, ultimately supporting the development of effective EHP management strategies in shrimp aquaculture.

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LINKING REARING CONDITIONS, MICROBIOME DEVELOPMENT AND RESILIENCE IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction:

The RAS system environment is a dynamic microbial system in which diverse microbial communities continuously interact with the host, influencing nutrient cycling, water quality, immune development, disease susceptibility, and overall fish performance. “Clean water” produced by UV and ozone sterilisation may disrupt affect microbiome development, potentially increasing vulnerability to later-life stressors. However, limited research has examined early-life microbial development and its long-term effects, highlighting a key knowledge gap in fish health and microbiome development. The aim of this study was to provide knowledge on the importance of the development of microbiome and water quality at egg, fry and alevin stages, and its effects on host health status. The resilience of the microbiome was also assessed in response to antibiotic intervention.

Methods:

A 15-week trial was conducted with rainbow trout from eyed eggs (270 degree days (DD)) to a final weight of 11.9 ± 0.96 g (1808 DD), followed by a 7-day oxytetracycline (OTC) treatment at 45 mg kg^{-1} . Eggs were reared in control recirculating aquaculture systems (RAS), RAS with ultraviolet treatment (RAS+UV), or RAS inoculated with faecal matter from healthy adult rainbow trout (RAS+FM), with four replicate systems per treatment and 200 eggs each. Water temperature was maintained at $11.3 \pm 0.5^\circ\text{C}$, DO at $8\text{--}9 \text{ mg L}^{-1}$, and flow at 3 L min^{-1} . Following exogenous feeding, fish were transferred to a common RAS and fed a commercial diet at 6% body weight daily. Water quality was maintained at $15.5 \pm 0.5^\circ\text{C}$, pH 6–7, and DO $8\text{--}9 \text{ mg L}^{-1}$, with ammonia, nitrite, and nitrate below 1.0, 0.1, and 30 mg L^{-1} , respectively. Sampling occurred at 270 DD (egg; S1), 493 DD (alevin; S2), 763 DD (fry; S3), and pre- and post-OTC fingerling stages at 1679 (S4) and 1808 DD (S5). Whole animals or intestines were sampled depending on developmental stage. Histology assessed goblet cell number and area, mucosal fold length, and lamina propria width. Parametric data were analysed using one- or two-way ANOVA with Tukey's *post-hoc* test, while non-parametric data were analysed using Kruskal–Wallis tests with Dunn's *post-hoc* test and Holm correction.

Results and Discussion:

Total Chemical Oxygen Demand TCOD during the initial egg microbial manipulation stage differed significantly among treatments ($p < 0.001$), with RAS+FM ($30.33 \pm 3.18 \text{ mg L}^{-1}$) higher than RAS ($21.08 \pm 2.22 \text{ mg L}^{-1}$) and RAS+UV ($22.00 \pm 2.81 \text{ mg L}^{-1}$), while RAS and RAS+UV did not differ ($p = 0.922$). No significant differences in growth, feed efficiency, survival, or deformities were observed (Table 1). Histology showed treatment-specific intestinal effects: before OTC, goblet cell area was greater in RAS than RAS+UV, while mucosal fold length and lamina propria width were greater in RAS+UV. Post-OTC, goblet cell area remained higher in RAS than RAS+FM, while RAS had the greatest lamina propria width; mucosal fold length differed ($p = 0.003$), with RAS+FM lower than RAS (Holm-adjusted $p = 0.00475$). OTC increased lamina propria thickness in RAS (Holm-adjusted $p < 0.001$), with no changes in RAS+UV or RAS+FM. Goblet cell numbers decreased in RAS+UV and RAS+FM, although the treatment $\times$ OTC interaction was not significant ($p = 0.478$). Overall, histological assessment indicated a less favourable intestinal health status in fish originating from the control RAS treatment following the antibiotic treatment. However, fish were transferred to a common RAS environment at the onset of exogenous feeding, reducing treatment-specific environmental and microbial exposure during subsequent development. Microbiome analysis of water communities and host-associated communities, and host gene expression profiling is in progress. These data will allow a greater understanding of the impact of the treatment effects during the experimental phase.

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(Continued on next page)

Treatment Group	RAS	RAS+FM	RAS+UV	<i>p</i> -value
Final Weight (g)	11.96±0.67	11.73±0.71	12.11±1.33	0.142
Mean Weight Gain (g)	11.32±1.27	11.26±1.51	11.1±1.05	0.193
Feed Conversion Ratio	0.98±0.1	0.95±0.04	0.96±0.1	0.994
SGR (% day ⁻¹)	3.3±0.18	3.52±0.1	3.4±0.14	0.889
Deformities* (%)	9.5±0.7	11.9±2.5	9.8±2.2	0.444
Survival Rate (%)	96.4±1.9	96.75±2.22	98±2.16	0.115

* determined during alevin stage

Table 1: Growth and production performance of rainbow trout fry during the 15-week trial after being reared in three different microbial environments.

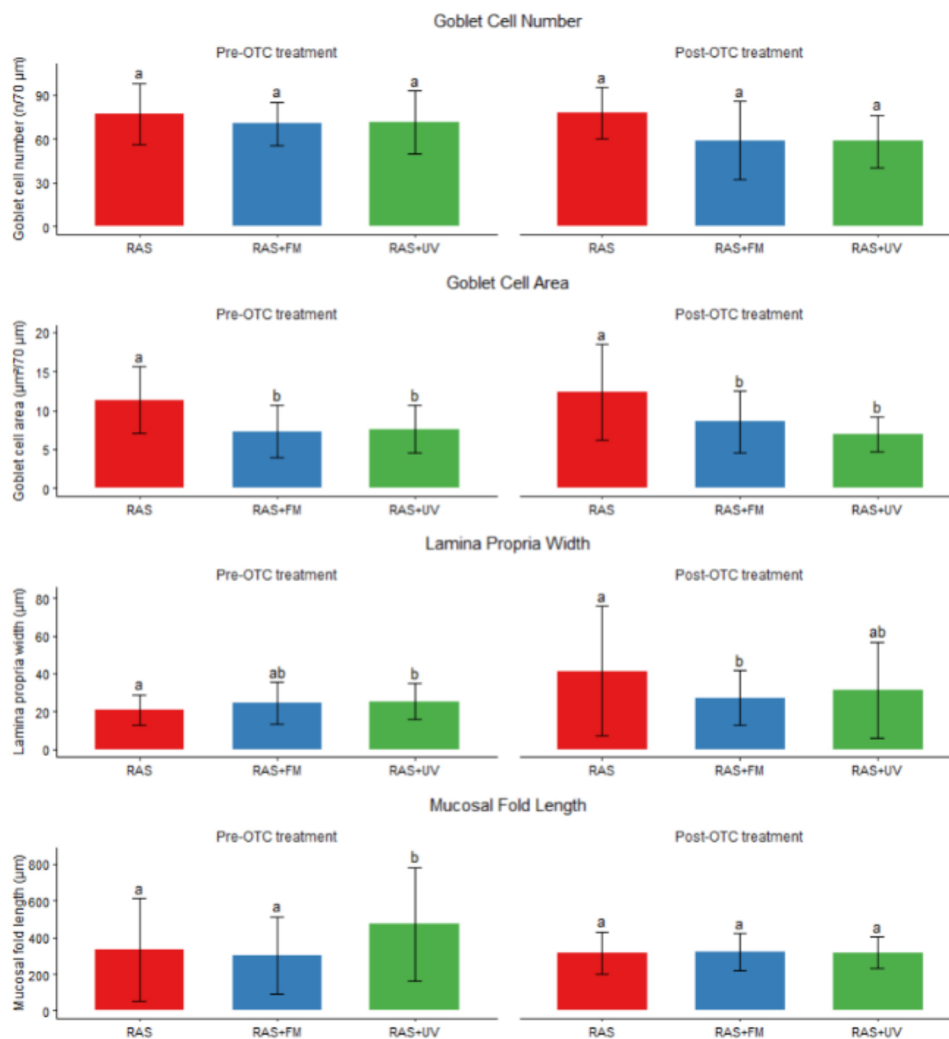


Figure 1: Posterior intestine morphometrics of rainbow trout fingerlings reared in the different systems after a 7-day OTC treatment. Bars with different superscripts signify significant differences among the means of the treatments.

ULTFARMS BELGIAN PILOT: LESSONS LEARNED AND MITIGATION STRATEGIES FOR OFFSHORE MULTI-USE AQUACULTURE

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The Belgian pilot of the ULTFARMS project explores the integration of aquaculture within offshore wind farms, building on earlier insights from the UNITED project. Conducted in the Belgian North Sea, the pilot combines seaweed cultivation, flat oyster farming, and nature-inclusive design approaches for oysters in a highly exposed offshore environment, aiming to develop scalable and resilient multi-use systems.

Despite significant environmental, technical, and logistical challenges, the pilot achieved key advancements in offshore aquaculture design and operation. Experimental work highlighted limitations in replicating offshore conditions, which were addressed through wave flume testing and scaled prototype validation. The development and deployment of submersible longline systems in a commercial field site demonstrated enhanced storm resilience, with successful submersion strategies informing buoyancy control and material durability. Field operations were frequently constrained by weather and vessel availability, necessitating adaptive approaches such as the reuse of existing datasets and flexible deployment strategies. Technical challenges in artificial oyster reef deployment emphasized the need for robust designs and innovative installation methods, including acoustic retrieval systems to overcome restrictions on scientific diving. Offshore cultivation trials further confirmed that early-stage deployment and seeding techniques are critical for success, while losses of cultivation units underscored the importance of material selection and structural optimization.

Financial and logistical constraints, including high insurance costs and limited offshore access, reinforced the importance of strong cross-partner collaboration and resource sharing. Mitigation strategies implemented throughout the project resulted in improved system resilience, refined design methodologies, and actionable insights for industry, academia, offshore wind operators, and policy stakeholders.

Key recommendations include the integration of contingency planning for extreme weather, dedicated budgeting for offshore risks, and prioritization of nearshore test systems as stepping stones toward full offshore deployment. Overall, the Belgian pilot demonstrates that while offshore multi-use aquaculture remains challenging, adaptive design, iterative testing, and collaborative approaches can significantly enhance feasibility and scalability.

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ACTIVE EUROPEAN FLAT OYSTER (*Ostrea edulis*) REEF RESTORATION IN A MARINE PROTECTED AREA, A BLUE CONNECT CASE STUDY IN THE BELGIAN DEMOSITE VLAAMSE BANKEN

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European flat oyster (*Ostrea edulis*) reefs formed extensive, biodiversity-rich habitats in the Belgian part of the North Sea until their collapse due to overfishing and disease. Within the Horizon Europe project BLUE CONNECT, an active restoration pilot is being implemented in the Vlaamse Banken Marine Protected Area, aiming to re-establish European flat oyster (*Ostrea edulis*) reefs where they historically occurred.

The Belgian demo site focuses on a carefully selected restoration location adjacent to a small shipwreck, situated within a marine reserve and Natura 2000 area. Site selection integrated historical oyster distribution data, high-resolution seabed substrate mapping, hydrodynamic assessments and extensive consultations with research, industry and governmental stakeholders. The restoration approach combines ecological realism with operational feasibility by using natural substrates that closely mimic native gravel beds of the Vlaamse Banken.

Certified *Bonamia*-free oyster larvae are remotely set on natural sedimentary rock substrates under controlled conditions before offshore deployment. Reef installation employs biodegradable bags and controlled drop-bag techniques, minimizing seabed disturbance.

An integrated monitoring framework accompanies the restoration, combining traditional diver-based surveys with innovative tools such as multibeam and side-scan sonar imaging and passive acoustic monitoring of reef soundscapes. In parallel, co-management principles are embedded through active stakeholder engagement, including local working groups and participatory tool validation.

This case study demonstrates how science-based site selection, restorative aquaculture, offshore engineering, innovative monitoring and stakeholder co-development can jointly support oyster reef restoration within marine protected areas, contributing to national and European restoration objectives.

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SOCIAL LICENCE TO OPERATE FOR LAND-BASED AQUACULTURE: DRIVERS OF PUBLIC OPPOSITION TO LARGE-SCALE RECIRCULATING AQUACULTURE SYSTEM PROJECTS

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As aquaculture expands to meet the growing demand for seafood, land-based recirculating aquaculture systems (RAS) are increasingly presented as a potentially more sustainable alternative to conventional marine cage farming. These systems offer several environmental advantages, including reduced water consumption, improved biosecurity, and limited interaction with wild fish populations. Nevertheless, several proposed large-scale RAS facilities in Europe and elsewhere have faced substantial public opposition resulting in project delays or cancellation.

This study examined why technologically innovative aquaculture projects with environmental ambitions may nevertheless encounter resistance. Three case studies involving proposed large-scale RAS facilities were compared. Each project generated debate among stakeholders, policymakers, environmental and animal-welfare organisations, and local communities. Information was collected from scientific literature, government reports, public inquiry documents, policy papers, media coverage and other publicly available sources. The cases were systematically compared to identify the principal concerns raised and the factors influencing project outcomes.

Animal welfare and environmental concerns - including wastewater discharge, energy and water use, and effects on the surrounding environment - featured prominently in all three cases. However, , landscape impacts, local community interests, trust in project developers, transparency, stakeholder engagement and governance also emerged as important sources of opposition. Importantly, the issues that dominated public debate were not necessarily the factors that ultimately caused projects to be delayed or cancelled. Project outcomes were shaped by a broader and interacting set of regulatory, governance, social and economic factors.

These findings demonstrate that technological innovation and environmental performance alone are insufficient to secure acceptance of large-scale land-based aquaculture. Developing and maintaining a social licence to operate also requires credible governance, transparent communication and meaningful stakeholder engagement. The study provides insights that can support the socially responsible development of future land-based aquaculture projects.

MICROPLASTICS IN RAS-REARED GIFT TILAPIA: POLYMER CHARACTERIZATION AND HAZARD INDEX ASSESSMENT

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Several studies have reported the accumulation of microplastics (MPs) in aquaculture environments, highlighting their potential adverse effects on aquatic ecosystems and their accumulation in aquaculture products, with possible implications for food safety and human health. This study aimed to evaluate the influence of plastic covers on the occurrence and characteristics of microplastics in a Recirculating Aquaculture System (RAS) used for rearing GIFT Tilapia over a 90-day production cycle. The experimental system consisted of eighteen 500-L tanks, including nine covered and nine uncovered units. Fish samples were collected at the beginning and at the end of the experiment to assess microplastic occurrence, polymer composition, and the associated Hazard Index. Gastrointestinal tracts (GIT) of 19 specimens were dissected and digested with H₂O₂ 35%, and incubated in an oven at 60°C for 48 h. They were then filtered through a glassfiber filter (GF/F), and retained particles counted under a Nikon stereomicroscope, using the software *ImageView*. The polymeric properties of MPs were analyzed using a Shimadzu IRXross Fourier Transform Infrared (FTIR) spectrophotometer equipped with an AIRSight microscope. FTIR spectra were acquired in transmission mode over the spectral range of 4000–700 cm⁻¹ with a spectral resolution of 8 cm⁻¹ and 100 accumulated scans. Only the samples with matching spectra above 70% and which exhibited the characteristic absorption bands of the identified polymer were considered. To assess the ecological harm of MPs, the Polymer Hazard Index (Lithner et al, 2011) and polymer hazard categories were calculated. After correction of number of MPs by confirmed particles in the FTIR, a total of 1932 MPs were registered in all GIT samples, with a mean of 101.7 particles/individual. Microplastic abundance did not differ significantly among treatments or maturation stages, and no significant treatment × maturation stage interaction was detected by two-way ANOVA ($p > 0.05$). Thus, the differences observed between sites with and without plastic cover fell within the range of variation associated with these non-significant effects. The order of MPs characterization in all samples analyzed by FTIR was Polyethylene-Kaolin composite (55.7%) > Polyacrylamide (13.4%) > Polyamide (11.5%) > Talc-Polyethylene composite (5.77%) > EVOH (3.85%), followed by Styrene-Butadiene, Polyester, PVC, Polycarbonate and Acrylic adhesive, all with 1.92%. A greater diversity of polymer types was observed in adult fish. Among the groups, the adult/covered and young/uncovered areas exhibited the highest and lowest diversities respectively. The Hazard Risk derived from the polymer characterization across all analyzed samples varied between 737 – 51267, indicating that both areas and maturation stages were above Health Risk Category III (High Risk), especially the Adult/Covered Area. The diversity of polymers identified further suggests that microplastic contamination in the system originates from multiple sources. While the precise origin of each polymer cannot be established, their occurrence is consistent with the weathering of aquaculture infrastructure, feed-related materials, plastic packaging, and other polymer-based products used during system operation. Thus, this study provides baseline data on microplastic contamination in tilapia reared under different cultivation conditions, supporting future investigations into contamination pathways and risk assessment in aquaculture systems.

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AGRONOMIC PERFORMANCE, MINERAL COMPOSITION AND BIOCHEMICAL CHARACTERISTICS OF BASIL *Ocimum basilicum* L. GROWN IN TROUT *Oncorhynchus mykiss* AQUAPONIC SYSTEMS

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Aquaponic systems are promising sustainable food production technologies that integrate fish culture and plant cultivation. Their performance depends on balanced environmental conditions for fish, nitrifying bacteria and plants. This study evaluated the agronomic performance, mineral composition and essential oil profile of basil grown in a trout-based decoupled aquaponic system. Two cultivation strategies were compared over 60 days: a non-supplemented system relying exclusively on trout-rearing water and a system supplemented with mineral nutrients formulated according to the Hoagland solution. Basil grown without mineral supplementation maintained a healthy appearance and stable physiological status, with satisfactory growth, although biomass remained lower than in supplemented plants. The mineral profile showed comparable nitrate and phosphorus concentrations in non-supplemented and supplemented plants, with nitrate levels of 5.40 ± 0.29 and 5.52 ± 0.29 mg g⁻¹, respectively, and phosphorus levels of 5.46 ± 0.23 and 6.14 ± 0.91 mg g⁻¹, respectively. In contrast, potassium concentration was lower in non-supplemented plants (36.89 ± 3.31 mg g⁻¹) than in supplemented plants (55.56 ± 7.16 mg g⁻¹). Essential oil yield per cultivated surface area remained comparable between systems, reaching 2.96 and 3.09 mL m⁻² in the supplemented and non-supplemented systems, respectively. GC-MS analysis showed that linalool (about 24%) was the predominant compound in both systems. Estragole content was markedly higher in non-supplemented plants ($21.35 \pm 1.46\%$) than in supplemented plants ($5.24 \pm 0.68\%$). These results show that trout-based decoupled aquaponics can support satisfactory basil production while enhancing essential oil accumulation and modulating aroma-related composition, making this approach a promising sustainable strategy for aromatic plant cultivation.

METABOLOMIC PROFILING REVEALS POTENTIAL BIOCHEMICAL DETERMINANTS OF EGG QUALITY IN ATLANTIC SALMON

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Introduction

Egg quality is a key determinant of reproductive success in Atlantic salmon (*Salmo salar*), yet the biochemical basis underlying its variation remains poorly understood. Variability in egg quality can significantly affect fertilisation success, embryo survival, hatching rate, and embryo viability, ultimately impacting production efficiencies (Bobe & Labbe, 2010; Brooks et al., 1997). In the past, egg quality in salmonids has been evaluated using post fertilisation outcomes which do not provide insight into the underlying biochemical processes that determine egg developmental competence (Aegerter & Jalabert, 2004; Lahnsteiner, 2000). Metabolomics has been applied to study fish responses to stress, nutrition, and disease, but its use in assessing egg quality remains limited. To the best of our knowledge, this is the first study to characterise the pre-fertilisation metabolomic profile of Atlantic salmon eggs, aiming to identify metabolites that distinguish egg batches by fertilisation success using a commercial Tasmanian population.

Materials and Methods

Freshly spawned unfertilised eggs were obtained from fifteen three-year-old Atlantic salmon females, and highly motile sperm was collected from two three-year-old neo-males at a Tasmanian hatchery in May 2024. Prior to fertilisation, subsamples were collected for metabolomic analysis. Each egg batch was fertilised in duplicate using sperm from both males and incubated individually in custom-made mini upwellers within a temperature and dissolved oxygen controlled freshwater recirculation system. Fertilisation success was assessed after three weeks of incubation based on neural streak formation. Eggs were subsequently freeze-dried, and metabolites were extracted using acetonitrile/methanol/water (2:2:1, v/v/v). Metabolite separation was performed using ultra-high-performance liquid chromatography (UHPLC), and annotation was conducted using a preliminary version of the Metabolite Annotation Propagation Synthesis (MAPS) pipeline (Cowled et al., 2026). Metabolic differences between high- and low-quality egg batches (defined by fertilisation success rate) were characterised using MetaboAnalyst 6.0 (Pang et al., 2024) to identify pathways associated with developmental competence.

Results and Discussion

A total of 1209 features were detected across egg samples, with 303 metabolites annotated in positive ion mode and 109 in negative ion mode. Low-quality eggs exhibited significantly higher levels of cyclic adenosine monophosphate (cAMP), spermine, D-camphor, and pivaloyltaurine. Pathway enrichment analysis reflected localised perturbations in amino acid metabolism and redox balance. These findings suggest the presence of metabolic stress, disrupted redox homeostasis, and changes in polyamine metabolism that may impair oocyte maturation and early embryonic development. High-quality eggs were characterised by increased levels of peptides and lipid-associated metabolites such as leucyl-prolyl-glycine, alanyl-valyl-proline isobutylamide, asparaginyllucine, glycyl-L-leucine, rhodanyl acetate, diphenyl sulfone, and clopidogrel. The enrichment of dipeptides and tripeptides may indicate enhanced amino acid availability for protein synthesis, while lipid-related metabolites suggest improved energy reserves and nutritional status.

Our study demonstrates distinct metabolic signatures associated with egg quality and fertilisation success in Atlantic salmon and propose potential biochemical markers for improving broodstock management and reproductive performance in Atlantic salmon aquaculture.

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NATURE-BASED NUTRIENT REMOVAL IN CARP POND AQUACULTURE USING WATERCRESS AND DUCKWEED

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Introduction

Semi-intensive common carp (*Cyprinus carpio*) pond aquaculture in Central and Eastern Europe provides valuable ecosystem services, including water retention and biodiversity support, yet also contributes to nutrient pollution and the risk of eutrophication. In 2022, European (EU27) carp production reached 63,865 t with a market value of nearly €190 million (FAO, 2024). Despite regulatory limits of 1,500 kg biomass ha⁻¹ (in Poland), effluent loading from semi-intensive systems can exceed the thresholds of the EU Water Framework Directive (EU-WFD, 2000) for total nitrogen (TN) and total phosphorus (TP). Phytoremediation — the use of aquatic macrophytes to remove nutrients from water — offers a promising, low-cost nature-based solution (NbS) that can simultaneously improve discharge quality and generate marketable biomass. Watercress (*Nasturtium officinale*) is a nutrient-dense, semi-aquatic perennial valued for its vitamins A, C, and K, as well as antioxidant and cancer-protective properties (Gill et al., 2007). Duckweed (*Lemna minor*) is a fast-growing floating macrophyte that contains up to 45% protein and is widely recognised for its efficient removal of nitrogen and phosphorus (Xu et al., 2023). The integration of both species into carp farm infrastructure has the potential to diversify farm income while mitigating eutrophication at scale (Eljasik et al., 2026).

Materials and Methods

The study was conducted at a commercial 120 ha (80 ha water surface) carp farm (Maliniec, northwest Poland, 53°42'N 15°21'E) producing approximately 28 t of carp annually in a traditional 3-year semi-intensive cycle. Four production systems were evaluated: watercress cultivated in (i) an unused earthen breeding pond (w-pond, 102.95 m²), (ii) a concrete discharge channel (w-channel, 169.93 m²), and (iii) a raft aquaponics system (w-aquaponics, 25.44 m²); and (iv) naturally established duckweed in the farm discharge channel (d-channel, 145.5 m²). Water quality parameters (pH, dissolved oxygen (DO), oxidoreduction potential (ORP), conductivity, total dissolved solids (TDS), and temperature) were measured in situ. Water samples were analysed for TN, nitrogen forms (NH₄⁺-N, NO₃⁻-N, NO₂⁻-N), TP, total reactive phosphorus (TRP), total carbon (TC), inorganic carbon (IC), and potassium (K). Harvested plant biomass was analysed for dry matter, ash, N, P, K, macro- and microelements, and heavy metals. Nutrient retention efficiency (%) was calculated as: (outflow conc. – inflow conc.) / inflow conc. × 100, following Kajgrova et al. (2024), and compared against EU-WFD thresholds for safe riverine discharge (≤3.5 mg TN L⁻¹; ≤106 µg TP L⁻¹).

Results and Discussion

Watercress achieved production yields of 11.92 ± 6.71 kg m⁻² (w-pond), 7.99 ± 0.99 kg m⁻² (w-channel), and 11.54 ± 3.73 kg m⁻² (w-aquaponics). Duckweed yielded 2.54 ± 0.77 kg m⁻² in the d-channel. TN was reduced by 5.93% (w-pond) and 8.63% (w-channel), while TP removal reached 23.43% via watercress (w-pond). Duckweed outperformed watercress in nutrient sequestration, removing 19.15% of TN and 57.89% of TP, along with significant reductions in NH₄⁺ (–44%), NO₃⁻ (–3.8%), NO₂⁻ (–13.8%), TRP (–68%), and organic phosphorus (–39.8%). Combined across all systems, the average retention relative to inflow was –9.9% for N and –25.6% for P, with negligible effect on carbon. All outflow waters met EU-WFD nitrogen limits; duckweed-treated water also approached compliance with phosphorus discharge thresholds. Both macrophytes remained within EU food-grade limits for heavy metals (Pb <0.30 mg kg⁻¹ w.w.; Cd <0.10 mg kg⁻¹ w.w.; As <20 µg kg⁻¹ w.w.), confirming their safety for human consumption (EC, 2023). Watercress nutrient uptake patterns varied across systems, reflecting the stoichiometric N:P ratio of the inflow water. TRP removal was observed in the w-pond (N:P ratio 40:1) but not in the w-channel (N:P ratio 11:1), consistent with the importance of nutrient stoichiometry for macrophyte phosphorus uptake. An unexpected increase in K concentration (+170.8%) in duckweed channel outflow was attributed to cation exchange, stress-induced K⁺ efflux, and leaching from decomposing biomass. The duckweed C:N:P ratio of 185:9:1 — shifted from the aquatic plant optimum of 119:17:1 (Hillebrand & Sommer, 1999) — indicates considerable remaining capacity for N and P removal under optimised harvesting regimes.

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Conclusions

Both watercress and duckweed demonstrated significant phytoremediation potential in effluents from semi-intensive carp aquaculture, with duckweed showing superior nutrient removal efficiency. Integration of these macrophytes into existing carp farm infrastructure (unused ponds, inter-pond channels, discharge channels) requires minimal investment while producing food- and feed-grade biomass that can diversify farm income. The multitrophic design further enhances the ecosystem services value of European fishponds, already estimated at $>1,257 \text{ € ha}^{-1}$ (Frélichová et al., 2014), and supports the water quality goals of the EU Water Framework Directive. Scaling these NbS across the Central and Eastern European aquaculture landscape could substantially reduce nutrient loads to inland waterways and help prevent eutrophication.

Acknowledgments

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STAKEHOLDER PERSPECTIVES ON EXPANSION POTENTIAL FOR BLUE MUSSEL AQUACULTURE IN THE NORTH ATLANTIC

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Introduction

Blue mussel farming is a form of non-fed aquaculture with high potential for more sustainable protein production, offering diverse ecosystem services while generally requiring fewer emissions and resources than fed aquaculture, such as salmon and other finfish (van der Schatte Olivier et al., 2020). There is growing interest in expanding this sector in response to calls for a transition towards sustainable and climate-smart protein to improve the health of the planet and people (Blanchard et al., 2017). Despite this potential, the global blue mussel aquaculture industry has remained relatively stagnant in recent decades (FAO, 2009). The potential for the expansion of the sector is currently influenced by supply chain efficiency (Krause et al., 2025; Avdelas et al., 2021), environmental conditions (Steeves & Filgueira, 2019; Padin et al., 2024), social acceptance (Wood & Filgueira, 2022), regulation (Fox et al., 2020), and economic conditions (Avdelas et al., 2021). These drivers generate uncertainties that hinder the industry's ability to plan for expansion by making it difficult to anticipate potential challenges and risks, and to identify where expansion opportunities may exist. Many drivers of mussel aquaculture are related to socio-economic and cultural contexts that vary geographically. Therefore, the expansion of mussel aquaculture will require coordinated yet place-specific strategic management to account for the many complex and interconnected external factors influencing the success of the sector. This study aims to identify the most relevant opportunities and barriers to the expansion of blue mussel aquaculture using semi-structured interviews with 18 stakeholders across 12 producer countries in the North Atlantic. This aim is addressed through a qualitative investigation and coding analysis using NVivo guided by the PESTEL (political, economic, social, technological, environmental, and legal) framework.

Results

Results revealed a greater number of barriers than opportunities across all PESTEL categories. Opportunities were identified but were often described as contingent on improvements in policy support, access to technology, and market development. Across all categories, findings highlight that mussel aquaculture is not constrained by a single limiting factor, but rather by interconnected structural challenges. Social factors emerged as the most prominent influence overall, particularly in relation to consumer demand and public acceptance, followed by environmental and economic factors. Technological factors were the only category more frequently associated with opportunities than barriers. Technology was also the least prominent category for barriers, suggesting that innovation may play a key enabling role. Legal factors related to government regulations were least frequently associated with opportunities, indicating an area where governments and policymakers could provide stronger support if expansion were a priority.

Discussion

This study offers fresh perspectives on the opportunities and barriers to blue mussel aquaculture expansion in the North Atlantic. Previous research has recognized key barriers, including low profitability, labour shortages, limited seed availability, environmental uncertainty, trade disruptions, low consumer demand, and complex licensing systems (Krause et al., 2025; Fox et al., 2020). Further, the participants in this study noted that climate change, restricted access to finance and technology, competition for marine space, and fragmented governance often affect small-scale producers most severely. Key opportunities recognized by the literature included sustainability, ecosystem services, contributions to food security, and rural employment (Avdelas et al., 2021; Bianchi et al., 2022). Participants also highlighted market opportunities through ecolabelling, tourism, geo-cultural branding, and product innovation, as well as technological advances in offshore farming, hatcheries, monitoring systems, and smart farming systems. Findings also showed apparent regional differences; for example, seed shortages were major concerns in some European countries but appeared less significant among participants from North America and Scandinavia.

Overall, the study suggests that successful expansion will require place-based strategies, streamlined regulation, market development, innovation, and coordinated policy that recognizes the role of mussel aquaculture in sustainable food systems, coastal economies, and environmental management.

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STAKEHOLDER PERSPECTIVES ON THE EXPANSION POTENTIAL FOR MUSSEL FARMING IN CANADA AND SWEDEN

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INTRODUCTION

Global blue mussel production reached approximately 132,000 tonnes in 2023, with Canada serving as the primary supplier to North America, producing roughly 21,989 tonnes annually (FAO, 2024). While the Canadian industry is well-developed and dominated by large-scale commercial entities, Sweden's sector is characterized by small-scale producers concentrated on the west coast, yielding approximately 1,684 tonnes annually (FAO, 2026). Growth in Sweden has been influenced by the Baltic Blue Growth project, which has promoted mussel farming primarily for nutrient removal and cycling, to support eutrophication mitigation rather than for direct human consumption. As global priorities shift toward expanding nutrient-dense, low-carbon protein sources, mussel farming represents an optimal pathway for addressing these goals (Bianchi et al., 2022). Given the differences in each country's approach to aquaculture, comparing their stakeholder perspectives is compelling because it may point to future directions for the blue economy.

METHODS

This study employs Q-methodology to identify and compare viewpoints among four stakeholder groups: industry, research, government, and NGOs. Thirty participants from Canada and Sweden took part in the study. Q-methodology combines qualitative and quantitative methods by using statistical techniques to examine subjective viewpoints. In environmental discourses, individuals often have conflicting viewpoints, beliefs, and experiences. Q-methodology studies are well-suited to identifying topics on which individuals agree by grouping responses based on shared priorities and concerns, ultimately finding areas of consensus and disagreement among groups of participants (Barry & Proops, 1999). The results of Q-methodology studies are groups of shared perspectives, qualitatively titled and described based on the observed interests of the group.

PRELIMINARY RESULTS AND DISCUSSION

Four dominant perspectives emerged from the dataset:

The Apprehensive Expansionist: Focused on commercial and economic potential, driven by market price and demand.

The Social-Acceptance Skeptic: Concerned with social and regulatory influences, such as misinformation and stakeholder conflicts.

The Eco-Innovator: Defined by an environmental focus on climate change adaptation and technological innovation.

The Production-Risk Pragmatist: Concerned with environmental threats and their impacts on production reliability and operating costs.

While "The Apprehensive Expansionist" perspective was represented across both countries, the other perspectives aligned primarily with one country. "The Social Acceptance Skeptic" is predominantly a Swedish perspective, while the "Eco-innovator" and the "Production-Risk Pragmatist" perspectives are primarily comprised of Canadian participants. This country-skewed composition of the perspectives suggests that viewpoints on expansion were not necessarily determined by stakeholder group alone, but that country-specific context may have shaped participant perceptions.

These Results suggest that market-related issues, such as demand projections and market price, remain universally relevant. In Sweden, findings indicate that clearer regulations and increased public awareness could facilitate the expansion of mussel farming. In Canada, the results reveal a strategic impasse between optimists (Eco-innovators) and risk-oriented (Production-Risk Pragmatists) perspectives, highlighting a tension that may stall future development.

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MYCOBIOME AND BACTERIAL MICROBIOTA OF MEAGRE (*Argyrosomus regius*) FED POULTRY BY-PRODUCT MEAL-BASED DIETS

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Introduction

Processed animal proteins, such as poultry by-product meal (PBM), combine high nutritional quality with a low environmental footprint, aligning with circular bioeconomy principles (Galkanda-Arachchige et al., 2020). Meagre (*Argyrosomus regius*), a high-value whitefish species native to the Adriatic Sea, has gained renewed interest in Mediterranean aquaculture due to its quality, rapid growth, and adaptability to farming conditions (Kružić et al., 2016). Beyond growth and economic performance, evaluating how alternative protein sources shape the gut microbial ecosystem is essential, since intestinal bacteria and fungi play key roles in digestion, nutrient absorption, and immune homeostasis, and can serve as sensitive early indicators of dietary impact on fish health. This study presents the first combined assessment of the bacterial (16S rRNA) and fungal (ITS2) intestinal communities of meagre fed increasing dietary inclusion levels of PBM as a fishmeal substitute.

Material and methods

Three experimental diets incorporating PBM as a partial fishmeal replacement at two inclusion levels (T1, T2), plus a fishmeal-based control diet (T3/CF), were formulated to meet the nutritional requirements of meagre. 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) were acclimated for four weeks on a commercial diet before being randomly distributed into six concrete tanks (600 L; 18 fish per tank, two replicate tanks per treatment). At the conclusion of the trial after one year, digesta content was sampled from the proximal (PI) and distal (DI) intestine of fish from each dietary group for gut microbial community characterization (12 replicates per treatment for each intestinal segment). Fungal (ITS2) and bacterial (16S rRNA V3-V4 region) community composition were characterized in parallel by amplicon sequencing on the Illumina platform. Sequences were denoised into amplicon sequence variants (ASVs) using DADA2 and taxonomically classified against the SILVA 138.2 (16S) and UNITE (ITS2) reference databases. Alpha and beta diversity were calculated for both datasets, and group differences were tested using ANOSIM and PERMANOVA (Bray-Curtis).

Results and discussion

The fungal (ITS2) community was diverse and highly individually variable, dominated by Malasseziomycetes, Dothideomycetes, Sordariomycetes, Eurotiomycetes and Saccharomycetes. Alpha diversity was consistently higher in the proximal than distal intestine, and the fishmeal control showed the lowest fungal diversity and evenness in both segments, while beta diversity and ANOSIM/PERMANOVA confirmed that dietary effects on the mycobiome were strongest distally, with T2 showing an intermediate community structure between T1 and the control.

The bacterial (16S) community was overwhelmingly dominated by Gammaproteobacteria across nearly all samples and diets, indicating a comparatively stable core bacterial assemblage relative to the fungal community. In contrast to the mycobiome, bacterial alpha diversity was highest, not lowest, in the fishmeal control, particularly in the distal intestine. Beta diversity, ANOSIM and PERMANOVA converged on a single, striking pattern: the distal intestinal bacterial community of the control group was markedly and significantly distinct from all other diet-by-segment combinations, whereas proximal communities and both PBM-fed groups (T1, T2) remained largely indistinguishable from one another regardless of gut segment.

Taken together, both microbial kingdoms indicate that PBM inclusion reshapes the meagre intestinal microbial ecosystem primarily in the distal intestine, but with contrasting directionality: PBM diets increased fungal diversity and evenness relative to the control, while the bacterial community of PBM-fed fish appeared more homogeneous than the compositionally distinct, more diverse distal bacterial community observed under the fishmeal-based diet. These complementary bacterial and fungal signatures underscore the value of a dual-kingdom amplicon approach for capturing diet-driven shifts in fish gut microbial ecology, and support poultry by-product meal as a viable, sustainable fishmeal alternative without inducing a uniformly destabilizing effect on the meagre gut microbiota.

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INVESTIGATION OF BREEDING STRATEGY FOR THE PRODUCTION OF LARGE TRIPLOID RAINBOW TROUT (*Oncorhynchus mykiss*) IN CONTEXT OF GLOBAL WARMING

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Introduction

Ongoing climate change is likely to profoundly alter aquaculture production conditions, particularly for freshwater species such as rainbow trout. Resilience, defined as an animal's ability to be minimally affected by disturbances or to rapidly return to its pre-disturbance state (Colditz and Hine, 2016), could represent a major focus in breeding objectives to help the sector cope with global warming. In addition, sterile triploid trout intended for smoked fillet production currently account for a large proportion of French production. The aim of this study was to evaluate the genetic parameters of resilience indicators between corresponding diploid and triploid trout families.

Material and methods

Three batches of 1,200 progenies each were derived from a single semi-factorial mating design of 100 females and 90 males from a commercial line of rainbow trout (*Oncorhynchus mykiss*) of the breeding company Les Sources de l'Avance. The first diploid batch (2n_breed) was reared in the breeding farm at stable temperature of 14°C. The two additional batches, one diploid (2n_prod) and the other triploid (3n_prod), were reared in a production farm characterized by a variable thermal profile with chronic episodes of elevated temperatures. Each individual was electronically tagged to monitor body weight four to five times until reaching the average body weight of 2.5 kg. Abiotic parameters (temperature, pH, etc.) were recorded throughout the experiment. At 2.5kg, individuals were slaughtered and measured for various processing traits. Fish were genotyped for parentage assignment using 1,000 SNP markers (Roche et al., 2024) sterile triploids are commonly used for production as sterility gives them potential gains in growth, yields, and quality. However, they cannot be reproduced, and DNA parentage assignment to their diploid or tetraploid parents is required to estimate breeding values for triploid phenotypes. No publicly available software has the ability to assign triploids to their parents. Here, we updated the R package APIS to support triploids induced from diploid parents. First, we created new exclusion and likelihood tables that account for the double allelic contribution of the dam and the recombination that can occur during female meiosis. As the effective recombination rate of each marker with the centromere is usually unknown, we set it at 0.5 and found that this value maximizes the assignment rate even for markers with high or low recombination rates. The number of markers needed for a high true assignment rate did not strongly depend on the proportion of missing parental genotypes. The assignment power was however affected by the quality of the markers (minor allele frequency, call rate. Animal models using pedigree kinship adapted to ploidy level were used for additive variance estimations. Genetic parameters were then estimated, including within-group genetic correlations and pairwise between-group genetic correlations across the two environments and the two ploidy levels.

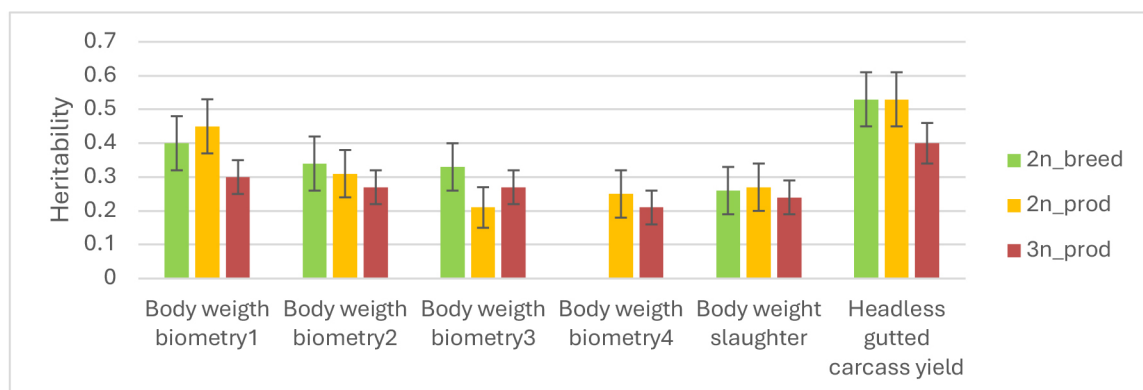


Table : Heritability of weights and headless gutted carcass yield according to batch (2n_breed in green, 2n_prod in yellow and 3n_prod in red)

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Results

Growth was faster at the breeding site than at the production site, allowing fish to be slaughtered at an average body weight of 2,127 g after 325 days post-hatching (dph) at the breeding site, whereas processing was carried out at 2,705 g for diploids and 2,575 g for triploids after 420 dph at the production site. Mortality reached 13.5% at the breeding site, compared with 27.8% for diploids and 34.2% for triploids at the production site. The difference in survival between 3n_prod and 2n_prod batches was significant during the early life stages but disappeared from 170 g (169 dph) onwards.

Headless gutted carcass yield, which is a proxy of fillet yield, at processing was 0.79 [0.01] for each batch, although the head yield is slightly higher at the production site, particularly for triploids (0.12 vs 0.11 vs 0.10, for 3n_prod, 2n_prod and 2n_breed respectively)

Heritability estimates for body weight ranged from 0.21 [0.05] to 0.45 [0.08], depending on the age at measurement, the rearing site, and ploidy level. They tended to decrease throughout fish growth. Genetic correlations for weight gain between ploidy levels at the production site were very high (>0.90), only estimation for weight gain during the autumn period was slightly lower (0.81 [0.28]). At slaughter, the genetic correlation between ploidy levels for body weight at slaughter was 0.73 [0.20], whereas that for headless gutted carcass yield reached 0.91 [0.06].

Comparison of weight gain and body weight for diploids across the two sites showed more moderate genetic correlations, ranging from 0.45 to 0.55 and 0.51 to 0.66 on comparable periods. The genetic correlation between sites for body weight of diploid fish at slaughter was 0.76 [0.17], and 0.87 [0.07] for headless gutted carcass yield. Genetic correlations between 2n_breed et 3n_prod were slightly lower.

Discussion

The results of this study highlight moderate genetic correlations for growth between the two rearing sites, suggesting that breeding strategies should be adapted according to the targeted breeding objectives for growth improvement. These differences may be partly explained by contrasting rearing conditions, particularly with regard to the thermal profiles of the farms. They become even more pronounced when the ploidy level of the breeding nucleus differs from that of the fish in production. However, genetic correlations for processing yields remained very high, suggesting no re-ranking of families for these traits according to ploidy or environment. This study provides a foundation for the development of breeding programs for diploid or triploid trout reared under contrasting environmental conditions.

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BEST OF BOTH WORLDS: NOR-GRAPE SUPPLEMENTATION LESSENS THE IMPACT OF VACCINATION ON GROWTH PERFORMANCE IN NILE TILAPIA (*Oreochromis niloticus*)

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Introduction

Despite its strong growth, disease remains a major constraint for aquaculture. With the intensification and multiplication of farms, disease has become a major constraint to the culture of tilapia. *Streptococcus agalactiae* is zoonotic and a leading cause of death (streptococcosis) in tilapia, quoted in the literature and by farmers and has been associated with infections in humans, related with the handling and preparation of infected fish. Vaccination is a key tool in improving farm protection against wild pathogens and their harmful impacts on fish health and mortality. However, vaccination events, due to their nature, can lead to momentaneous reduction of growth performance, while fish are mounting their immune response. The aim of this experiment was to evaluate the effect of Nor-Grape, a dry grape extract feed additive, for its potential to attenuate drawbacks of vaccination on growth performance in tilapia.

Materials and Methods

375 juvenile tilapias (16.6g±0.3g) were randomly distributed in 15 100L tanks (25 fish per tank) and fed for 4 weeks with 2 distinct experimental diets: a control diet (CTL, COPPENS) and a supplemented diet (NG, CTL diet + 70g Nor-Grape®/T). After one week of feeding, fish were subjected to an intra-peritoneal vaccination through injection at 0,1ml with a *S. agalactiae* vaccine (VAC). A CTL group was not vaccinated (NEG) to evaluate the impact of vaccination on growth. Feeding was continued for another 3 weeks post-vaccination. Mortality was assessed daily. Growth (Initial body weight and final body weight, weekly weight gain, specific growth rate SGR) and feed utilization (FCR) were also monitored.

Results and discussion

Fish growth was significantly impacted by the vaccination event in the CTL VAC group compared to the CTL NEG fish ($p<0.05$). However, no difference on growth performance was observed between NG fish and the CTL NEG group ($p>0.05$) showing that the supplementation lessened the impact of the vaccination on growth.

Thus, Nor-Grape can prove a useful supplementation in fish around vaccination to limit its impact on growth to get the best of both worlds: biosecurity and optimal growth.

DIETARY GREEN TEA EXTRACT INCLUSION FOR TURBOT POSTLARVAE: EFFECTS ON GROWTH, REDOX STATUS, AND MOLECULAR RESPONSE

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Introduction

Marine fish larvae are particularly vulnerable to oxidative stress and metabolic imbalances during early ontogeny. The intensification of marine larviculture needs innovative strategies to enhance larval robustness and resilience. Green tea extract, rich in polyphenols such as epigallocatechin gallate (EGCG), has emerged as a promising phytobiotic due to its potent antioxidant and health-promoting properties. In species like turbot (*Scophthalmus maximus*), maintaining gut integrity and metabolic efficiency is crucial for successful weaning and subsequent performance. Incorporating botanical extracts may provide a non-invasive tool to prime the larval physiological system, potentially improving the response to common acute stressors. While the antioxidant benefits of green tea are well-documented in adult fish, its role in modulating the metabolic and molecular pathways during the fast-growing postlarval stage remains poorly understood. This study aims to evaluate the potential of green tea extract as a functional dietary supplement to strengthen turbot postlarvae, assessing its impact on growth performance, redox physiology, and the molecular regulation of key metabolic pathways before and after an acute thermal challenge.

Material and Methods

The trial was conducted using turbot (*Scophthalmus maximus*) postlarvae reared in a Recirculating Aquaculture System (RAS) from 29 to 51 days after hatching (DAH). Three experimental diets were tested in triplicate: a commercial-like control (CTRL) and two diets supplemented with green tea extract at two inclusion levels, LOW and HIGH (the latter being double the former). At 51DAH, larvae were subjected to an acute thermal shock of +5°C above the rearing temperature, followed by a second sampling. Growth performance KPIs, including dry weight, survival, total length, and relative growth rate (RGR), were recorded. Physiological redox status was assessed alongside molecular analyses. Gene expression related to redox regulation (*sod2*, *gpx4*, *cat*, *nrf2*), nitrogen metabolism (*gdh*, *glul*, *got2*), amino acid transporters (*pept1*, *lat1*), digestive capacity (*tryp*), lipid metabolism (*aco*, *fasn*, *fabp2*), gut integrity (*cldn15*, *tjp1*, *muc2*), mitochondrial biogenesis (*atp5f1a*, *ppargc1a*, *opa1*), and cellular stress (*hsp60*, *hsp70*, *sirt3*) was quantified.

Results

No significant differences were observed in survival, dry weight, total length, or RGR across treatments. Before the thermal challenge, SOD activity was lower in green tea-fed groups compared to the CTRL. Following the temperature stressor, the *gdh* gene was significantly upregulated in the LOW treatment relative to the CTRL, although *glul* and *got2* remained unaffected. Regarding the redox response post-challenge, *sod2* and *gpx4* showed no differences, while *cat* and *nrf2* expression was lower in the LOW group compared to the CTRL. Other markers for cellular stress, digestive capacity, lipid metabolism, gut integrity, and mitochondrial biogenesis were not significantly affected by the diets or the stressor.

Conclusions

Dietary inclusion of green tea extract did not alter turbot growth performance but successfully modulated key molecular pathways. The upregulation of *gdh* under thermal stress suggests a higher nitrogen metabolism adjustment during environmental challenges, with further mobilization of amino acids as energy source. Crucially, the downregulation of *nrf2* and *cat* likely reflects a direct ROS scavenging effect by green tea polyphenols, exerting a ‘sparing effect’ on the endogenous antioxidant system by reducing the need for gene activation. Overall, green tea extract shows potential to bolster metabolic resilience and physiological priming in marine larviculture.

Acknowledgments

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FROM MOUTH TO INTESTINE: LONG-TERM FEEDING EFFECTS OF INCREASING SOYBEAN PROTEIN CONCENTRATE LEVELS IN EUROPEAN SEABASS *Dicentrarchus labrax* REARED IN RAS

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Introduction

A growing demand for seafood and the ensuing environmental effects are pushing the need for innovation in aquaculture and a critical examination of technological advancement in several key areas, such as novel feed composition and feed technologies. The increasing need for aquafeeds and limited fish meal supply has led to alternative ingredients in feed formulations. This may result in higher carbohydrate content in diets, affecting fish digestion and environmental waste production. As known, soy protein is the major source of protein as fishmeal replacement in fish feed because of its worldwide availability and low price. However, to our knowledge there is a lack of information regarding the use of X-ray microtomography to evaluate change in microstructure of feed blends added different concentration of soybean protein concentrate (SPC). Nonetheless, significant knowledge gaps persist regarding the long-term physiological and nutritional effects of these protein sources in carnivorous species maintained in RAS. The aims of the study, therefore, were to investigate the pellet's characteristics (bulk density, expansion ratio, diameter and pellet's porosity) and their usage properties (oil leakage rate, durability, hardness and floatability) and to assess growth performance, digestibility, gut microbiome profile of European sea bass fed with experimental diets containing increasing levels of SPC over 16 weeks long-term feeding.

Material and Methods

Juvenile European seabass were obtained from a local hatchery and transferred to a marine RAS until reaching an initial mean weight of 32 g. Fish were stocked in a fully controlled RAS with 15 tanks (250 L capacity; 30 fish per tank). Five iso-nitrogenous (48% CP) and iso-lipidic (18% CL) extruded diets were formulated, including a control diet with low fishmeal content. Soybean Protein Concentrate replaced fishmeal at 25% (SPC25), 50% (SPC50), 75% (SPC75), and 100% (SPC100) levels. Fish were hand-fed twice daily to apparent satiety during acclimation and throughout the 16-week experimental period. Micro-CT analysis was performed using a Skyscan 1275 X-ray microtomograph. Faeces were collected via passive settlement systems attached to each tank outlet during the final 30 days of the trial. Total RNA was extracted from intestinal samples using TRIzol reagent according to the manufacturer's protocol. RNA quality and quantity were assessed using spectrophotometric and fluorometric methods. Raw paired-end FASTQ files were quality-checked using FastQC (v0.12.1) and MultiQC (v1.21). Taxonomic profiling was conducted using a Naïve Bayes classifier in QIIME2 trained on the SILVA 138 SSU rRNA database (99% identity, 338F/515R region). Diversity analyses were performed using QIIME2 core-metrics and the phyloseq package (v1.44) in R.

Results

Overall, increasing SPC inclusion leads to a progressive transition from a compact and uniform structure to a more porous and heterogeneous matrix, particularly for SPC75, and SPC100. This trend suggests that higher SPC levels negatively affect pellet densification and cohesion, likely influencing key functional properties such as mechanical strength, water stability, and hydration behavior. Percent porosity (total, open, closed), mean cell size (MCS) and cell size and cell wall thickness will be presented in detail. No significant differences in growth, feed intake, and feed conversion ratio were observed among the experimental diets, further indicating that high levels of SPC inclusion had a negligible effect on overall growth performance. Collectively, while dry matter digestibility remained unaffected, increasing dietary inclusion of SPC significantly reduced both protein and lipid digestibility. The reduction was more pronounced at higher inclusion levels, particularly in the SPC75 and SPC100 groups, suggesting that elevated SPC incorporation may negatively impact nutrient availability and digestive efficiency in fish. With respect to microbiome analysis, mean Shannon diversity ranged

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from 1.85 (SPC0) to 2.26 (SPC75), with no significant differences among groups. Samples generally clustered without clear dietary separation; however, outliers were observed, including samples 6323 and 6324 (SPC50) along the negative PC1 axis and sample 6329 (SPC100) along the positive PC1 axis. The gut microbiota was dominated across all dietary groups by Firmicutes, Proteobacteria, Actinobacteriota, and Bacteroidota. At the genus level, the most abundant taxa were *Lactobacillus*, Ruminococcaceae (unclassified), *Pseudomonas*, and *Photobacterium*. Genus-level abundances showed high inter-individual variability, consistent with elevated intra-group beta diversity dispersion.

Discussion

The present study showed that, although no significant differences in growth performance were observed, pellets with high Soybean Protein Concentrate inclusion exhibited a clear transition from a dense, well-integrated matrix to a highly porous and structurally heterogeneous structure, indicating reduced pellet integrity at higher inclusion levels. The host genotype appears to exert a stronger influence on gut microbiota composition than diet, which may partly explain the observed outliers, potentially reflecting genetic variability or differential sensitivity to SPC anti-nutritional factors. Individual differences in intestinal transit time, mucus composition, and innate immune status may also contribute to these microbiome deviations under high SPC dietary stress. The numerical increase in ASV richness in high-SPC groups (SPC75, SPC100) may be associated with greater availability of plant-derived substrates, such as complex polysaccharides and secondary compounds, potentially supporting a wider range of microbial taxa capable of metabolizing these compounds in the intestine of European seabass. Further investigation is required to clarify the functional implications of these observations.

Acknowledgement

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GROWTH, FEED EFFICIENCY, AND NUTRIENT UTILISATION EFFICIENCY OF MEAGRE (*Argyrosomus regius*) REARED AT 20 °C

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Introduction

Meagre is an emerging candidate for diversification of Mediterranean aquaculture because of its rapid growth, fillet quality and adaptability to production conditions. However, species-specific information for defining feeding strategies remains limited. Accurate feeding is important to sustain growth and feed efficiency while limiting unnecessary feed use and nutrient losses. In meagre, feed-intake models have been proposed (Azevedo et al., 2025), and protein and energy requirements were also estimated (Jauralde et al., 2021). This study assessed growth performance, feed utilization, whole-body composition, and feed intake associated with zero biomass gain of meagre juveniles reared at 20 °C.

Materials and methods

Meagre (*Argyrosomus regius*) juveniles (initial body weight 43.2 g; 20 fish per tank) were distributed among 12 tanks and fed a diet formulated to target 50% crude protein and 16% crude lipid on a dry-matter basis at 0, 12.5, 25, 50, 75, or 100% of apparent satiation intake for 31 days ($n = 2$ tanks per feeding level). The two tanks assigned to 100% were hand-fed twice daily until feeding activity ceased and one or two pellets remained uneaten. Their mean daily feed intake was recalculated weekly and used to establish the restricted feeding levels for the following week, with tank allowances adjusted to the number of fish present. Growth performance, survival, feed utilisation, and whole-body dry matter, lipid, ash, and gross energy were analysed. Data were analysed by one-way analysis of variance followed by Tukey's test ($P < 0.05$). Feed efficiency and FCR used mortality-adjusted biomass gain. Relative feed intake and biomass gain were expressed per kg mean mortality-adjusted tank biomass per day. A quadratic-plateau model described growth, and a quadratic model described FCR for tanks with positive gain. Zero-gain intake was estimated from the growth model. Protein intake was estimated from the formulation target and measured feed dry matter (95.60%); gross energy intake used measured dietary energy (20.79 kJ g⁻¹ as-fed).

Results and discussion

Final body weight, weight gain, and specific growth rate increased with feeding level (all $P < 0.001$). Final body weight reached 58.4 and 60.2 g at 75 and 100% feeding, respectively, with no significant difference between these levels. Survival increased from 55.0% at 0% feeding to 100% at 75–100% feeding ($P = 0.003$). Feed efficiency peaked at 0.96 at 75% feeding and declined to 0.75 at 100% ($P = 0.001$). The quadratic-plateau growth model ($R^2 = 0.993$; $n = 12$) predicted zero biomass gain at 3.03 g kg⁻¹ day⁻¹ (Figure 1), equivalent to 20.7% of the apparent *ad libitum* feed intake by interpolation between the 12.5% and 25% treatment mean intakes. The plateau onset (14.96 g kg⁻¹ day⁻¹) was beyond the observed intake range. The quadratic FCR model had $R^2 = 0.941$ ($n = 8$). At 75% feeding, mean feed intake was 10.01 g kg⁻¹ day⁻¹, corresponding to an estimated protein intake of 4.79 g kg⁻¹ day⁻¹ and gross energy intake of 208.2 kJ kg⁻¹ day⁻¹; corresponding intakes at predicted zero gain were 1.45 g protein kg⁻¹ day⁻¹ and 62.9 kJ kg⁻¹ day⁻¹. Whole-body dry matter differed among feeding levels ($P = 0.003$), with the lowest mean at 0% (18.09%) and the highest at 100% feeding (24.7%). Values at 0% were lower than at 25–100%, and values at 12.5% were lower than at 50–100%. Whole-body lipid was also affected ($P = 0.003$), with the lowest and highest means at 0% (0.75%) and 100% feeding (3.75%), respectively. Lipid was lower at 0–12.5% than at 50–100%, while 25% was intermediate. Whole-body gross energy differed among feeding levels ($P = 0.004$), with the lowest mean at 0% (3.03 kJ g⁻¹) and the highest at 100% feeding (5.14 kJ g⁻¹). Values at 0% were lower than at 50–100%, and values at 12.5% were lower than at 75–100%, while 25% was intermediate. Ash was unaffected by feeding level ($P = 0.692$). All composition values are expressed on a fresh-matter basis. The lower lipid content and energy density under restricted feeding are consistent with reduced body energy reserves, but do not quantify nutrient retention.

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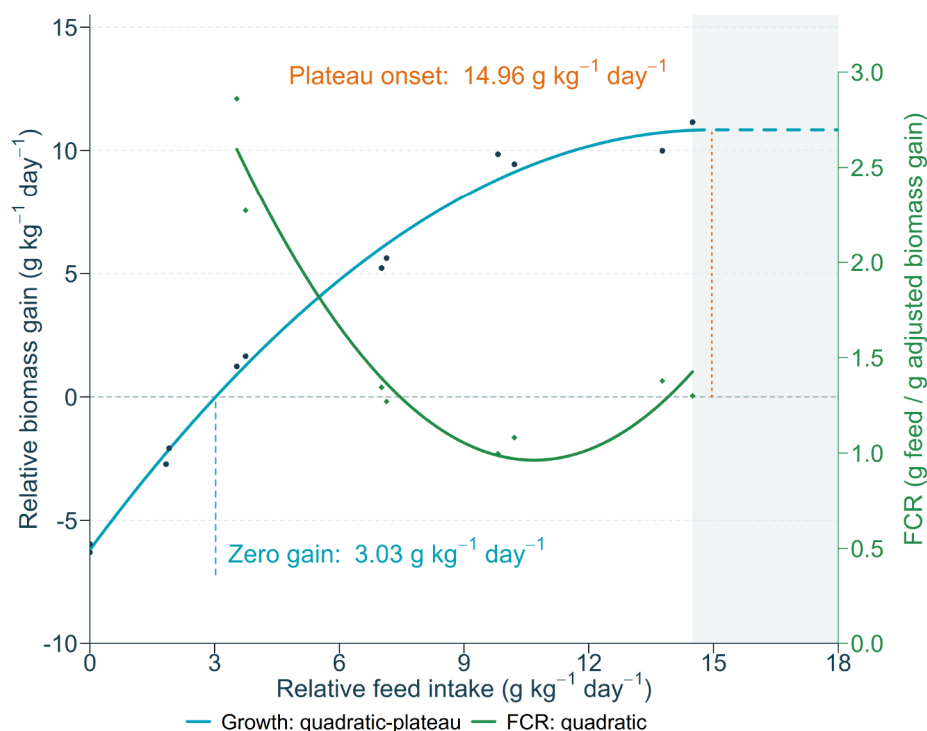


Figure 1. Relative biomass gain and FCR versus relative feed intake at 20 °C. Growth: quadratic-plateau model (left axis); FCR: quadratic model (right axis). Points represent individual tanks. Dashed lines indicate predicted zero gain ($3.03 \text{ g kg}^{-1} \text{ day}^{-1}$) and plateau onset ($14.96 \text{ g kg}^{-1} \text{ day}^{-1}$); shading denotes extrapolation. Relative intake and gain use mean mortality-adjusted tank biomass.

Conclusion

Overall, feeding juvenile meagre at approximately 75% of apparent satiation intake was sufficient to sustain growth performance while improving feed utilization under the experimental conditions. Zero biomass gain was predicted at $3.03 \text{ g kg}^{-1} \text{ day}^{-1}$ at 20 °C. The 75% treatment corresponded to approximately $10 \text{ g feed kg}^{-1} \text{ mean biomass day}^{-1}$. Increasing the ration to full satiation reduced feed efficiency without a statistically detectable increase in final body weight.

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CHARACTERIZATION OF THE INNATE IMMUNE SYSTEM OF *Penaeus kerathurus*: A FIRST APPROACH

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Introduction

Shrimp aquaculture has expanded rapidly over the last decades, becoming one of the fastest-growing sectors of global aquaculture. However, infectious diseases continue to be a major constraint, causing an estimated loss of nearly 40% of global production capacity and resulting in billions of dollars in economic losses each year. Viral pathogens account for a substantial proportion of these losses (Flegel & Sritunyalucksana, 2011; Underwood et al., 2013). A better understanding of the immune mechanisms of *Penaeus kerathurus*, including its cellular responses and molecular pathways, is essential for improving disease management and supporting the sustainable development of Mediterranean shrimp aquaculture. As crustaceans rely exclusively on innate immunity, mediated by both cellular and humoral components, elucidating these defense mechanisms is crucial for the development of effective, non-antibiotic health management strategies (Aguirre Guzman et al., 2009; Chong et al., 2022) because their culture is limited by the development of infectious diseases. As invertebrates, shrimp's natural immunity acts as a fast and efficient defence mechanism against the pathogens. Their immune system involve hemocytes (for encapsulation, nodule formation and phagocytosis). Therefore, the aim of this study was to characterize the humoral immune parameters of the hemolymph of *Penaeus kerathurus* and establish baseline reference values that will facilitate future immunological studies, health assessment, and the development of standardized protocols for this emerging aquaculture species.

Material and methods

Twenty wild *Penaeus kerathurus* specimens were collected with the collaboration of the Mar Menor Fishermen's Guild (Murcia, Spain) and maintained in experimental aquaria under controlled conditions ($25 \pm 1^\circ\text{C}$, salinity 35‰, continuous aeration, and appropriate substrate to promote animal welfare and natural behaviour). All individuals were morphometrically measured and sexed prior to sampling. Shrimp were anesthetized following established protocols (Rotllant et al., 2023), and hemolymph samples were collected individually under aseptic conditions. Samples were immediately stored at -80°C until subsequent analysis of humoral immune parameters.

The total protein content of the hemolymph was determined using the Bradford protein assay (Bradford, 1976). In addition, the activity of several enzymes involved in the humoral immune response was evaluated using spectrophotometric assays following established protocols. Peroxidase activity in the hemolymph was determined according to the method described by Quade and Roth, with some modifications (Quade & Roth, 1997). Protease and antiprotease activities were measured in hemolymph following the protocols described with some modifications (Hanif et al., 2004; Ross et al., 2000).

Results and discussion

The analysis of hemolymph revealed measurable levels of total protein together with detectable peroxidase, protease, and antiprotease activities in all specimens, demonstrating the suitability of these biomarkers for evaluating the humoral immune status of *Penaeus kerathurus*. Although individual variability was observed among animals, the measured values were consistent with the expected physiological range reported for healthy penaeid shrimp.

These findings provide the first baseline characterization of humoral immune parameters in *Penaeus kerathurus*, representing a valuable reference for future studies on nutrition, health, environmental stress, and disease resistance. Establishing standardized reference values will facilitate the interpretation of immune responses under experimental and farming conditions, contributing to the development of this emerging Mediterranean aquaculture species.

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IMMUNOMODULATORY POTENTIAL OF A SUSTAINABLE PLANT CELL CULTURE-DERIVED *Rosmarinus officinalis* EXTRACT IN GILTHEAD SEABREAM (*Sparus aurata* L.) LEUKOCYTES: AN IN VITRO STUDY

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Introduction

Global aquaculture has expanded rapidly to meet the increasing demand for animal protein, but intensive farming practices often compromise fish health and immune competence, while the extensive use of antibiotics has promoted environmental contamination and antimicrobial resistance (Dawood et al., 2022; Orso et al., 2022). Consequently, sustainable alternatives such as plant-derived bioactive compounds are gaining attention. Rosemary (*Rosmarinus officinalis*) has been reported to enhance growth, antioxidant capacity and immune responses in several fish species, although inconsistent results regarding dosage and administration methods have limited its application (Abdel-Tawwab et al., 2024; Kolygas et al., 2025). Plant cell suspension cultures represent a sustainable source of standardized bioactive compounds, including rosmarinic acid, a metabolite with well-known antioxidant and antimicrobial properties (Bulgakov et al., 2012; Hakkim et al., 2019). Therefore, the aim of the present study was to evaluate the in vitro immunomodulatory effects of an elicited rosemary cell suspension culture extract on head kidney (HK) leukocytes of gilthead seabream (*Sparus aurata* L.), a key marine aquaculture species.

Material and methods

Rosemary cell suspension cultures were established from friable calli in liquid MS medium and elicited with 75 μ M methyl jasmonate. Phenolic compounds were extracted from lyophilized biomass using an ethanol:acidified water mixture and characterized by HPLC. Five specimens (442.6 $\pm$ 190.9 g) of gilthead seabream, were kept for two weeks in re-circulating seawater aquaria from Marine Resources Centre of IMIDA (San Pedro del Pinatar, Spain). The water temperature was maintained at 20.1 $\pm$ 2.0 °C with a flow rate of 900 L h⁻¹ and 35‰ salinity and 12 h light: 12 h dark photoperiod. Animals were killed after starving for 24h by using a overdose of clove oil. All experimental protocols were approved by the Ethical Committee of IMIDA and CEEA UPV/EHU (N° A13260703). HK leukocytes were isolated according to (Espinosa et al., 2018) and adjusted to 1 $\times$ 10⁷ cells mL⁻¹. Then, cells were incubated for 24 h with rosemary extract (0, 0.1, 1, 10 and 100 μ g mL⁻¹), and cell viability, phagocytic activity and respiratory burst were evaluated following established protocols (García Beltrán et al., 2020).

Results and discussion

Exposure to the rosemary cell suspension culture extract did not significantly affect HK leukocyte viability, indicating the absence of cytotoxic effects at the tested concentrations. Similarly, phagocytic activity remained unchanged after 24 h of incubation. However, phagocytic capacity was significantly enhanced at 10 μ g mL⁻¹, whereas it decreased at 100 μ g mL⁻¹. Likewise, respiratory burst activity increased significantly at 0.1 μ g mL⁻¹ but was significantly reduced at 10 and 100 μ g mL⁻¹. These findings suggest a dose-dependent immunomodulatory effect, in agreement with previous studies reporting that rosemary-derived bioactive compounds can stimulate innate immune responses at low concentrations, whereas excessive doses may exert inhibitory or pro-oxidant effects (Abdel-Tawwab et al., 2024; Kolygas et al., 2025). Overall, the results indicate that elicited rosemary cell suspension culture extract is a promising sustainable source of immunomodulatory compounds for aquaculture, although optimization of the effective dosage will be essential before its application as a functional feed additive.

Acknowledgment

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IN VITRO ASSESSMENT OF *Morus alba* LEAF EXTRACT ON THE IMMUNE RESPONSE OF GILTHEAD SEABREAM (*Sparus aurata* L.) LEUKOCYTES

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Introduction

Morus alba, commonly known as white mulberry, has been shown to possess significant anti-inflammatory properties, primarily through the inhibition of leukocyte migration and modulation of inflammatory pathways (Chen et al., 2013). Besides, *Morus alba* leaf extract exhibits lipolipidemic, antiviral, antimicrobial, neuroprotective effects (Aghdam et al., 2024), together with a strong antioxidant activity, which is beneficial in reducing oxidative stress, increasing glutathione levels and decreased nitrite/nitrate and malondialdehyde levels (Dkhil et al., 2015). This makes it a promising candidate for enhancing the immune response in fish, such as the gilthead seabream, which is a species of economic importance in aquaculture.

Material and methods

Fresh *Morus alba* leaves were collected from a selected genotype maintained in the IMIDA Germplasm Bank (BAGERIM), freeze-dried, and milled into a fine powder. A hydroethanolic extract (70% ethanol) was prepared using orbital shaking and ultrasonic-assisted extraction. The extract was centrifuged, filtered, concentrated by rotary evaporation to remove ethanol, freeze-dried, and stored at -80°C until use in the in vitro assays. Five gilthead seabream (*Sparus aurata*) (mean body weight: 442 ± 190 g) were maintained for two weeks in recirculating seawater systems at the Marine Resources Centre of IMIDA (San Pedro del Pinatar, Spain) under controlled environmental conditions ($20.1 \pm 2.0^{\circ}\text{C}$, 35‰ salinity, 900 Lh⁻¹ water flow, and 12 h light: 12h dark photoperiod). Following a 24 h fasting period, fish were euthanized by an overdose of clove oil. All procedures were conducted in accordance with protocols approved by the Ethical Committee of IMIDA and the CEEA UPV/EHU (No. A13260703). Head kidney leukocytes were isolated as previously described by (Espinosa et al., 2018) and adjusted to a final concentration of 1×10^7 cells mL⁻¹. Cells were exposed for 24 h to different concentrations of *Morus alba* extract (0, 0.1, 1, 10 and 100 $\mu\text{g mL}^{-1}$). After incubation, cell viability, phagocytic activity and respiratory burst were assessed using previously established methodologies (García Beltrán et al., 2020).

Results and discussion

Incubation of HK leukocytes with *Morus alba* leaf extract for 24 h did not compromise cell viability at any of the concentrations tested, demonstrating that the extract was not cytotoxic under the experimental conditions. Phagocytic activity was also unaffected by treatment, whereas phagocytic capacity showed a significant reduction at the highest extract concentration. In contrast, respiratory burst activity was significantly enhanced at 1 $\mu\text{g mL}^{-1}$ but decreased markedly following exposure to 100 $\mu\text{g mL}^{-1}$ revealing a concentration-dependent effect on leukocyte function. These results are consistent with previous reports showing that low concentrations of aqueous or ethanolic botanical extracts, including oregano and other medical plants, can stimulate innate immune responses such as phagocytosis and respiratory burst in fish leukocytes, whereas higher concentrations may attenuate these responses (Beltrán et al., 2018; Camara-Ruiz et al., 2020; Fazio et al., 2017). Overall, *Morus alba* leaf extract appears to be a promising natural source of bioactive compounds for aquaculture, although further studies are required to determine the optimal concentrations for its potential use as a functional feed additive.

Acknowledgment

AP acknowledges the financial support received from the European Regional Development Fund (ERDF/FEDER) through the ERDF Operational Programme of the Region of Murcia (2021-2027), Project No. 50463 “Development of Sustainable Models of Agricultural, Livestock and Aquaculture Production” (Subproject: Innovation in the Field of Sericulture: New Materials, Biomaterials and Extracts of Biomedical Interest). CER was funded by a grant of the Ramón y Cajal Fellowship Programme from the Spanish government (RYC2023-045252-I). The authors would like to thank all the technical staff of the Marine Resources Centre of IMIDA (San Pedro del Pinatar, Spain) for their valuable support and assistance throughout this study.

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EVOLUTION OF THE MUSCLE ELEMENTAL PROFILE DURING ATLANTIC BLUEFIN TUNA (*Thunnus thynnus*) FATTENING AT TWO COASTAL SITES

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Introduction

The elemental composition of Atlantic bluefin tuna muscle (ABFT, *Thunnus thynnus*) may vary during the fattening phase and between coastal sites. Its analysis makes it possible to characterize the evolution of the elemental profile from capture and identify common patterns among fattening areas. The objective was to evaluate these changes by comparing tuna sampled immediately after capture with tuna maintained for three and five months in cages located at two coastal sites in Spain.

Materials and methods

Seventy-four ABFT muscle samples were analyzed and distributed into five groups: wild specimens caught in the Balearic Sea ($n = 14$) and tuna maintained for 3 and 5 months at two coastal sites in the Region of Murcia, southeastern Spain: San Pedro del Pinatar (SPP) and El Gorguel, Cartagena (EG) ($n = 15$ per group). The concentrations of Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P, Se, S and Zn were determined using optical ICP, and differences between groups were evaluated (Kruskal-Wallis test). Elements showing significant differences were included in a principal component analysis (PCA) and a canonical discriminant analysis (CDA).

Results and discussion

Significant differences among groups were detected for Ca, Cu, K, Mg, Mn, Na, P and S, whereas Cr, Fe, Se and Zn did not vary significantly (Table 1). However, when recently caught specimens were compared with those maintained for five months, most of the elements studied showed no significant changes. The most relevant variations were observed in Ca and Na, whose concentrations decreased during the fattening period, and in Mn, which increased markedly, particularly at the final sampling.

The PCA retained three components, which explained 77.4% of the total variance. The first was mainly defined by K, Mg and S; the second by Ca, Na and P; and the third by Cu and Mn. Partial differentiation was observed between freshly caught specimens and those maintained at the fattening facilities. The CDA, based on K, Mg, Mn, Na and Cr, separated the five groups (Figure 1) and correctly classified 84.4% of the samples and 75.7% after cross-validation.

Overall, the muscle elemental profile varied during fattening, mainly because of decreases in Ca and Na and an increase in Mn at both sites. The classification obtained suggests that the multielement composition could differentiate newly caught and fattened specimens.

Conclusion

Fattening slightly modified the muscle elemental profile of *T. thynnus* at both sites with particularly evident changes in Ca, Na, and Mn. The multivariate differentiation observed suggests an effect of fattening time and a possible contribution of site to muscle elemental composition. These differences could be related to diet, although specific studies would be required to confirm this hypothesis.

Acknowledgment

To the Ricardo Fuentes Group company for facilitating the sampling of tunas

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Table 1. Median values of the muscle elemental profile according to origin and fattening time.
The same letter indicates statistically significant differences between the groups sharing it (Kruskal-

Group	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	P	Se	S	Zn
Wild	0.014 ^{a,b}	0.079	0.309 ^a	14.500	0.253 ^a	0.018	0.384 ^{a,b}	0.075 ^{a,b,c}	0.181 ^{a,b}	0.216	0.144	6.830
SPP-3 m	0.004 ^a	0.079	0.256	10.668	0.195 ^a	0.017 ^a	0.369 ^{c,d}	0.029 ^a	0.152 ^a	0.210	0.117 ^a	8.359
EG-3 m	0.009	0.038	0.219 ^{a,b}	11.123	0.220	0.019	0.388 ^{e,f}	0.041	0.170	0.236	0.140	8.390
SPP-5 m	0.004 ^b	0.001	0.309 ^b	17.503	0.235	0.019	2.283 ^{a,d,f}	0.027 ^b	0.172	0.140	0.147 ^a	9.663
EG-5 m	0.005	0.414	0.241	16.360	0.219	0.021 ^a	2.109 ^{b,c,e}	0.034 ^c	0.159 ^b	0.001	0.137	9.243

Wallis; $p < 0.05$). Ca, K, Mg, Na, P and S: g 100 g⁻¹; others: mg kg⁻¹ (fresh weight).

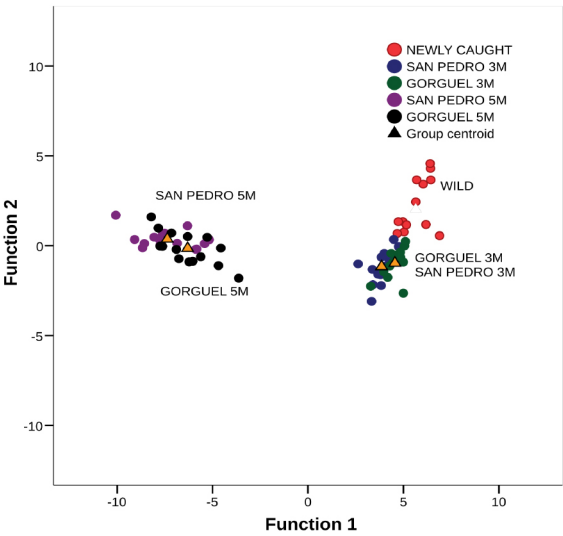


Figure 1. Discriminant analysis according to origin and fattening time.

EFFECT OF FATTENING ON THE MUSCLE FATTY ACID COMPOSITION OF ATLANTIC BLUEFIN TUNA

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Introduction

Fatty acids play important roles in fish physiology and contribute to the nutritional value of their tissues. Among them, long-chain n-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are of particular nutritional interest. During fattening, lipid accumulation may be accompanied by changes in the fatty acid composition of muscle. The aim of this study was to evaluate the evolution of the muscle fatty acid profile of Atlantic bluefin tuna (*Thunnus thynnus*) from capture to 3 and 5 months of fattening at two coastal sites in the Region of Murcia.

Material and methods

A total of 74 muscle samples from *T. thynnus* were analyzed and distributed among five groups: wild specimens captured in the Balearic Sea (n = 14), and tuna maintained for 3 and 5 months at San Pedro del Pinatar (SPP) and El Gorguel (EG), both located in the Region of Murcia, southeastern Spain (n = 15 per group). The relative composition of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), including n-3 and n-6 fatty acids, was evaluated, together with their concentrations on a fresh-weight basis. The n-3/n-6 ratio and the combined EPA+DHA concentration were also calculated. Differences among groups were assessed using ANOVA followed by the Games–Howell post hoc test. Fatty acid concentrations were additionally included in a principal component analysis (PCA) and canonical discriminant analysis (CDA).

Results

The fatty acid profile changed during fattening, with a similar pattern observed at both sites. After 5 months, the proportion of SFA decreased, whereas PUFA and, particularly, n-3 fatty acids increased at both SPP and EG.

The n-3/n-6 ratio also increased during fattening, from 3.6 in wild specimens to 8.1 and 8.3 after 5 months at SPP and EG, respectively. Similarly, the EPA+DHA concentration increased from 0.110 g 100 g⁻¹ in wild specimens to 4.724 g 100 g⁻¹ at SPP and 4.792 g 100 g⁻¹ at EG after 5 months, representing an increase of more than 40-fold compared with the initial value. The simultaneous increase in both the proportion and concentration of n-3 fatty acids indicates a substantial modification of the muscle lipid profile during fattening.

The PCA performed using fatty acid concentrations explained 93.0% of the total variance and showed a separation of samples mainly associated with fattening duration. The CDA correctly classified 100% of the samples, with 98.6% correct classification after cross-validation. These results demonstrate the high discriminatory capacity of quantitative fatty acid composition for differentiating the groups studied, while the overall pattern of fatty acid evolution was similar at both locations.

Conclusions

Fattening markedly modified the muscle fatty acid profile of *T. thynnus*, with an increase in n-3 PUFA and EPA+DHA concentrations, particularly after five months at both SPP and EG. The greater abundance of these nutritionally relevant fatty acids reinforces the value of Atlantic bluefin tuna muscle as a source of long-chain n-3 fatty acids. Furthermore, the quantitative fatty acid profile showed a high discriminatory capacity among the groups studied.

Acknowledgements

To the Ricardo Fuentes Group company by facilitating the sampling of tunas.

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Table 1. Median values of the muscle elemental profile according to origin and fattening time.
The same letter indicates statistically significant differences between the groups sharing it (Kruskal-

Group	Ca	Cr	Cu	Fe	K	Mg	Mn	Na	P	Se	S	Zn
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SPP-3 m	0.004 ^a	0.079	0.256	10.668	0.195 ^a	0.017 ^a	0.369 ^{c,d}	0.029 ^a	0.152 ^a	0.210	0.117 ^a	8.359
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SPP-5 m	0.004 ^b	0.001	0.309 ^b	17.503	0.235	0.019	2.283 ^{a,d,f}	0.027 ^b	0.172	0.140	0.147 ^a	9.663
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Wallis; $p < 0.05$). Ca, K, Mg, Na, P and S: g 100 g⁻¹; others: mg kg⁻¹ (fresh weight).

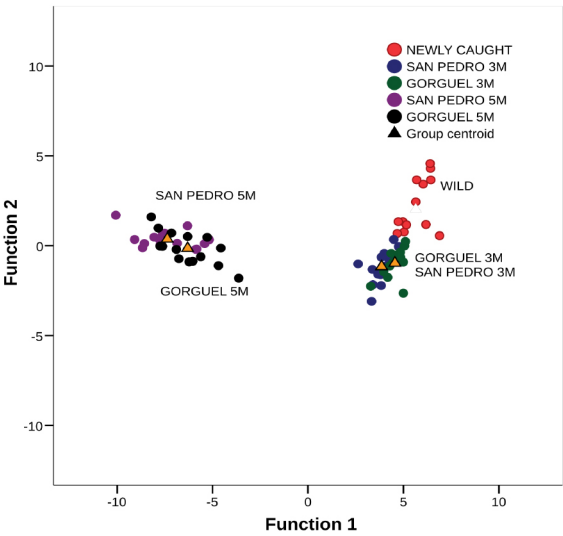


Figure 1. Discriminant analysis according to origin and fattening time.

COMPARISON OF REDOX BIOMARKERS IN PLASMA AND SKIN MUCUS OF ATLANTIC BLUEFIN TUNA, *Thunnus thynnus*

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Introduction

Atlantic bluefin tuna (*Thunnus thynnus*) is a pelagic species with high swimming activity, in which maintaining the balance between oxidants and antioxidants is relevant for preserving cellular integrity and physiological function. In fish, skin mucus constitutes one of the first lines of defence against the marine environment and is continuously exposed to oxygen, microorganisms, and other potentially oxidising agents. Plasma, in turn, provides information on systemic processes in the organism. The joint analysis of both matrices makes it possible to assess whether they provide similar or complementary information on the oxidant-antioxidant balance. The aim of this study was to compare the concentrations and associations of five redox biomarkers in plasma and skin mucus from wild adult *T. thynnus* specimens.

Materials and methods

Plasma and skin mucus samples were collected from 16 adult *T. thynnus* specimens caught in the Barbate tuna trap (Cádiz, Spain). In both matrices, five redox biomarkers were determined: cupric ion reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP), Trolox equivalent antioxidant capacity (TEACH), total thiols, and advanced oxidation protein products (AOPP). Plasma and mucus concentrations were compared using Student's *t*-test. Correlations between matrices, among biomarkers within each matrix, and between their concentrations and body weight were also evaluated.

Results and discussion

All biomarkers showed significantly higher concentrations in skin mucus than in plasma (Table 1). Differences ranged from 1.9- to 3.5-fold for CUPRAC, FRAP, TEACH, and thiols, whereas the AOPP concentration was 21.2-fold higher in mucus. The simultaneous increase in antioxidant capacity indicators and AOPP, as a marker of protein oxidation, suggests that mucus exhibits more intense local redox activity than plasma.

No significant correlations between plasma and mucus were detected for any biomarker. Within each matrix, seven of the ten possible correlations were significant in plasma ($r = 0.503\text{--}0.984$), whereas all ten were significant in mucus ($r = 0.616\text{--}0.988$). The presence of significant associations among all biomarkers in mucus indicates that antioxidant capacity and protein oxidation indicators varied in a more coordinated manner in this matrix. This pattern could be related to its direct exposure to the external environment and the local interaction between oxidant production and antioxidant defences at the body surface. In plasma, by contrast, biomarkers may be influenced by processes originating in different organs and tissues, which could explain less generalised covariation. Nevertheless, these correlations do not demonstrate that all biomarkers share the same regulatory mechanism.

The absence of correlations between the two matrices suggests that mucus does not directly reflect the systemic redox status observed in plasma. Body weight was not correlated with any of the biomarkers evaluated.

Conclusion

Skin mucus showed greater antioxidant capacity and a higher AOPP concentration than plasma, suggesting more intense local redox activity. The absence of correlations between the two matrices, together with the greater covariation of biomarkers in mucus, indicates that plasma and mucus represent distinct physiological compartments. Therefore, mucus should not be considered a direct substitute for plasma, although it may constitute a complementary and less invasive matrix for assessing local antioxidant and oxidative processes in wild adult Atlantic bluefin tuna.

Acknowledgements

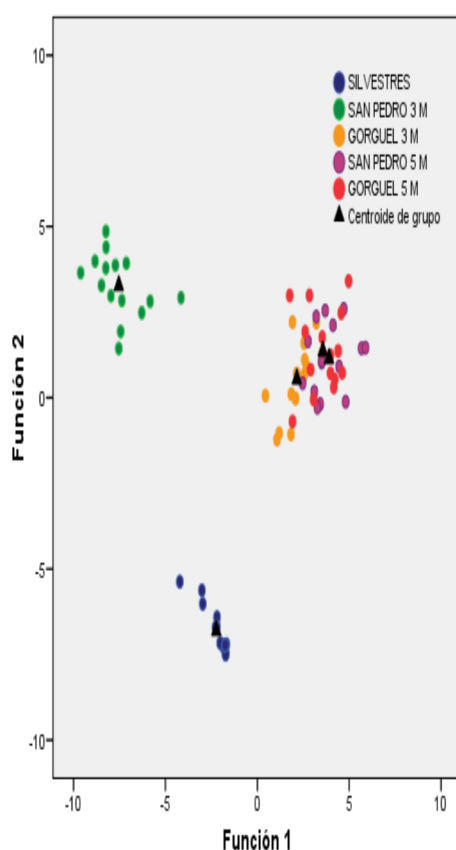
To the Ricardo Fuentes Group company by facilitating the sampling of tunas.

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Table 1. Main indicators of the muscle fatty acid profile during fattening.

Groups	SFA (%)	PUFA (%)	n-3 (%)	n-3/n-6	EPA+DHA (g 100 g ⁻¹)
Wild	31,6 ± 7,0 ^{a,b}	14,9 ± 9,7 ^{a,b}	11,7 ± 7,8 ^{a,b}	3,6 ± 1,0 ^{a,b,c}	0,110 ± 0,057 ^{a,b,c,d}
SPP-3 m	30,6 ± 2,7 ^{c,d}	12,2 ± 6,2 ^{c,d}	9,7 ± 5,6 ^{c,d}	4,7 ± 2,0 ^{d,e}	1,631 ± 1,290 ^{a,e,f}
EG-3 m	29,3 ± 2,7 ^{e,f}	14,2 ± 4,0 ^{e,f}	11,5 ± 3,7 ^{e,f}	5,7 ± 1,3 ^{a,f,g}	1,870 ± 0,778 ^{b,g,h}
SPP-5 m	24,4 ± 0,7 ^{a,c,e}	26,7 ± 1,2 ^{a,c,e}	22,9 ± 1,1 ^{a,c,e}	8,1 ± 0,5 ^{b,d,f}	4,724 ± 0,675 ^{c,e,g}
EG-5 m	24,7 ± 0,9 ^{b,d,f}	25,7 ± 2,3 ^{b,d,f}	22,1 ± 2,1 ^{b,d,f}	8,3 ± 0,6 ^{c,e,g}	4,792 ± 0,754 ^{d,f,h}

Data are presented as mean ± SD. Different letters indicate significant differences among groups (ANOVA, Games–Howell; $p < 0.05$). SFA: saturated fatty acids; PUFA: polyunsaturated fatty acids; SPP: San Pedro del Pinatar; EG: El Gorguel; EPA+DHA: g 100 g⁻¹ fresh weight.

**Figure 1.** Canonical discriminant analysis based on fatty acid concentrations.

CASCADE EXTRACTION OF COLLAGEN-BASED PROTEIN FROM ATLANTIC COD FILLETING SIDE STREAMS

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Introduction

Atlantic cod (*Gadus morhua*), part of the Gadidae family, makes up about 10% of all finfish caught globally, with an annual production of around 0.7 million tonnes. The main processing method is filleting, which yields only 33% of the fish's total weight as commercial product (Falch et al., 2005). The remaining side streams (head, backbone, and skin) constitute roughly 34% of the raw fish mass and are rich in collagen, the most abundant protein in vertebrate tissues. Depending on the extraction conditions, collagen may be obtained as its native triple helix form, as gelatin, or as partially hydrolyzed peptides. Each fraction has distinct functional and biological properties, enabling a variety of industrial applications. However, most research focuses on extracting only one fraction at a time. In this study, we employed a rapid cascade extraction process using green technologies to sequentially recover native collagen, gelatin, and peptides from Atlantic cod skin, head, and backbone.

Material and Methods

SINTEF (Norway) provided skin, head, and backbone from Atlantic cod. The frozen tissue was sent to Chalmers University of Technology in Gothenburg, Sweden, where it was ground into pieces about 2 cm in size using an electric meat grinder and stored at -20 °C. The cascade extraction began with an alkaline pretreatment using 0.1 M NaOH to remove non-collagenous proteins. These proteins were then isolated through isoelectric precipitation and freeze-dried. Next, collagen was extracted with 0.5 M acetic acid by ultrasound at 80% amplitude for 30 minutes. The collagen was obtained by salting out with NaCl, followed by dialysis and freeze-drying. The residual material from the native collagen extraction was mixed with hot distilled water and incubated with stirring to extract gelatin. The water temperature during gelatin extraction was set at 45 °C for skin and 60 °C for head and backbone. Finally, the residue from head and backbone obtained from gelatin extraction was further extracted with subcritical water extraction at 170 °C for 20 minutes. The yield was calculated relative to the initial mass of each side stream on a dry basis, and its chemical composition was characterized.

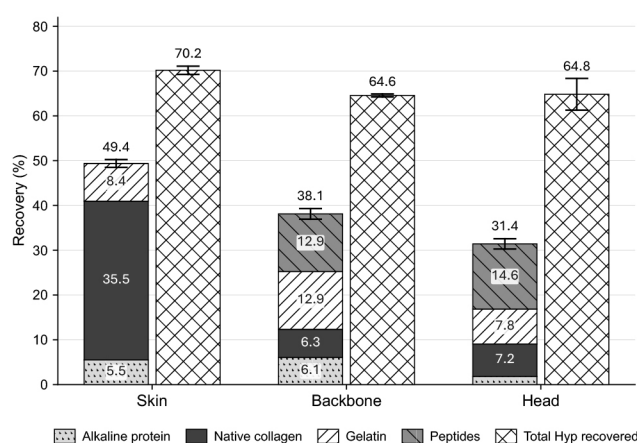


Figure 1. Cascade extraction yields from Atlantic cod skin, backbone, and head. Stacked bars show each fraction as % of raw material dry matter, with the total above; the adjacent bar shows total hydroxyproline recovered (% of that present in the raw material). Means $\pm$ SD, n = 2.

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Results

The composition results reveal a notable difference in the initial biomass makeup. Protein content in the head and backbone ranges from 37.8% to 43.9%, with minerals as the dominant component (45.0-52.1%) in these tissues. Conversely, the skin shows a high protein level (74.0%) and a more moderate mineral content (20.3%). Lipids are a small fraction in all tissues, not exceeding 4%. Figure 1 illustrates the extraction yields from each Atlantic cod tissue. The skin yields the most native collagen, followed by gelatin. For the head and backbone, subcritical water yielded the most, followed by gelatin and then collagen. The total mass recovered is higher in the skin than in the backbone and head, but the hydroxyproline recovery percentage is similar across tissues. Regarding protein content, recovered proteins after alkaline pre-treatment exceeded 60% in all tissues. Collagen from the skin had protein levels over 93%, while in the head and backbone, it was considerably lower at 44% and 27%. The same trend was observed in gelatin, with 91% protein in skin and about 80% in the head and backbone. Lastly, peptides from the head and backbone showed protein contents above 90% in both cases.

Discussion

The cascade extraction process yielded 50% protein from Atlantic cod skin. Although the total yield from the head and backbone was lower, minerals make up nearly half of their dry weight, greatly reducing the organic matter available for extraction compared with the skin. The percentage of hydroxyproline recovered provides a better perspective on the extracted collagen proteins and is similar across all three tissues. On the other hand, the difference in protein levels in the collagen of the head, backbone, and skin is due to the fact that during the acid extraction of collagen, the minerals that are part of the head and backbone are partially solubilized. While this process decreases the purity of the extracted collagen, it makes it easier to extract gelatin and peptides later, as shown by the higher protein recovery in these steps.

Acknowledgment

This work was supported by Formas – a Swedish Research Council for Sustainable Development (grant number:) funding the FOODIMAR project. Additionally, the authors want to extend their gratitude to the partners of the FOODIMAR project: SUBMARINER Network for Blue Growth EEIG (DE), SINTEF Ocean AS (NO), TETIS Biotechnology (TR), University of Southern Denmark (DN) and Ghent University (BE) for the valuable input over the project.

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INSECT EXUVIAE MEAL AS AN ALTERNATIVE TO EXISTING INSECT-BASED MEALS: ASSESSMENT OF TWO SCREW-PRESSING TECHNIQUES FOR DEFATTING BLACK SOLDIER FLY LARVAE

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Introduction

Projections indicate a 10% increase in aquaculture production in the next decade (FAO, 2026). To support this, securing feed ingredients is crucial. However, recent climate and geopolitical challenges have increased the cost and reduced the availability of conventional ingredients. Black soldier fly larvae meal (*Hermetia illucens*, BSFL), recognized for its high nutritional value and ability to be reared from organic waste, has emerged as a promising substitute. BSFL contains 30% to 40% lipids on a dry basis, with over half being saturated fatty acids. Defatting boosts protein content and reduces the negative effects of lipids on the meal's sensory qualities and shelf life. Mechanical hot pressing is a widely used defatting method. Although the process involves dried larvae, studies on undried larvae are limited and mainly concentrate on soft-tissue fractions; the exuviae portion is often discarded. In this study, we evaluated the yield, composition, and *in vitro* digestibility of meals produced by mechanically pressing dry and fresh BSFL.

Materials and Methods

Defatted BSFL meal was prepared via dry mechanical pressing (DPD-BSFL), in which dried larvae were screw-pressed at 140 °C. For wet mechanical pressing (WPD-BSFL), the undried larvae were screw-pressed at 140 °C, and the exuviae fraction was oven-dried at 65 °C. The chemical composition of the BSFL meals was determined according to the methods described by the Association of Official Analytical Chemists (AOAC, 2005). For the *in vitro* digestibility assay, enzymatic extracts from three species involved in marine and freshwater aquaculture were used: red seabream (*Pagrus major*), rainbow trout (*Oncorhynchus mykiss*), and Nile tilapia (*Oreochromis niloticus*). Acid and alkaline protease activity was analyzed, and the EA:PI ratio (protease activity to dietary protein intake) was calculated for each species and extract. The *in vitro* digestibility assay included a gastric phase followed by an intestinal phase. During each phase, enzymatic activity was calibrated according to the EA:PI ratios, and digestibility was assessed by measuring the free amino groups released. Non-defatted BSFL meal (ND-BSFL), fish meal, and soybean meal were included in the *in vitro* digestibility trial.

Results

DPD-BSFL showed higher yield ($p < 0.05$) than WPD-BSFL, producing 55% versus 36% meal (dry basis). Its defatting efficiency was also superior ($p < 0.05$), with an 84% reduction in lipid content versus 80% in WPD-BSFL. In terms of composition, no significant differences were found in lipid and protein contents between the two defatted meals. WPD-BSFL had higher chitin levels ($p < 0.05$), while DPD-BSFL had higher ash levels. The EA:PI results (Figure 1A) indicated that rainbow trout displayed the highest activity of acid and alkaline proteases relative to ingested protein. The *in vitro* digestibility of soybean meal was lower than that of the other meals across all three species. No significant differences were observed in defatted BSFL and non-defatted meals digestibility among these species ($p < 0.05$), although Nile tilapia showed significantly higher fish meal digestibility compared to the other meals.

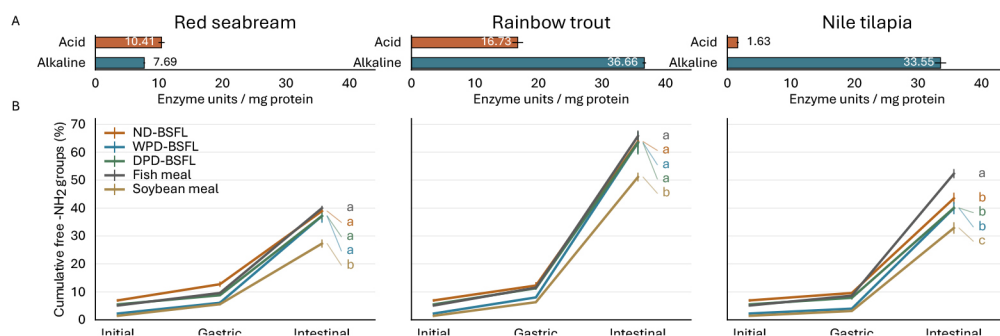


Figure 1. EA:PI ratio for acid and alkaline protease (A) and cumulative *in vitro* release of free -NH₂ groups across digestion stages (B) in three fish species. Means $\pm$ SD, $n = 3$. Within each species, meals sharing a letter do not differ (Tukey HSD, $\alpha = 0.05$).

(Continued on next page)

Disussion

This study shows that WPD-BSFL and DPD-BSFL are effective defatting methods, both producing meals with less than 10% fat and over 60% protein. Moreover, the WPD process yields an additional fraction consisting of BSFL internal tissues and lipids, which can be further processed to extract lipid and protein components that have potential as functional food ingredients (Kang et al., 2024). The *in vitro* digestibility of BSFL meals with red seabream and rainbow trout digestive extracts was similar to that of fish meal. In Nile tilapia, however, the lower digestibility of BSFL meals might result from the species' omnivorous habit, leading to decreased gastric enzymatic activity and predominantly intestinal digestion. The intestinal phase is the most significant stage for amino group release across all species. However, despite the EA:PI ratio of intestinal protease in Nile tilapia being four times higher than in red seabream, no increase in the final digestibility of BSFL meals was observed. This suggests that, although the gastric phase has a lower proteolytic hydrolysis capacity, it plays an important role in influencing digestibility during the intestinal phase.

Acknowledgment

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DIETARY PHYTO AQUANITY IMPROVES FEED EFFICENCY AND ANTIOXIDANT STATUS IN NILE TILAPIA : IN VIVO AND IN SILICO INSIGHTS

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Introduction

Improving feed efficiency while maintaining physiological robustness is a critical goal for sustainable tilapia production. Botanical bioactive compounds mainly curcuminoids and Artepilin C have attracted interest among for their potential antioxidant, immunomodulatory and anti-inflammatory activities and their ability to support fish performance [1]. This study assessed the effect of dietary Phyto AquaNity (PAN), a standardized botanical mixture of curcuminoids from turmeric and Artepilin C from green propolis, on feed efficiency and antioxidant status of Nile tilapia (*Oreochromis niloticus*) in rearing conditions. Moreover, molecular docking was used to explore the potential mechanism of action of curcumin based on antioxidant markers.

Materials and methods

Juvenile tilapia with initial body about 73g were reared in 2m³ happas for 80 days. Fish supplemented with control diet were compared with fish receiving a mixture of botanical compounds (Phyto AquaNity, PAN at 0.5 g.kg⁻¹). Growth performance, feed utilization parameters and antioxidant markers were assessed. In addition, a molecular docking was performed with curcumin against three proteins associated with oxidative stress regulation such as : kelch-like ECH associated protein 1 (Keap1), 15 lipoxygenase (15-LOX) and xanthine oxidase (XO).

Results and Discussion

Over trial duration, PAN significantly improved feed utilization through the reduction of feed conversion ratio from 1.46 ± 0.03 (control fish) vs 1.24 ± 0.03 (supplemented fish) group while protein efficiency ratio increased from 2.25 ± 0.05 to 2.69 ± 0.08 ($p < 0.05$). Although growth parameters were not significantly improved, weight gain and specific growth rate were numerically increased compared to the control group. PAN significantly enhanced antioxidant responses through the increase of antioxidant enzymes mainly superoxide dismutase (29.93 ± 2.06 vs 39.64 ± 1.41 mU mL⁻¹), glutathion peroxidase (3.47 ± 0.12 vs 4.51 ± 0.07 mU mL⁻¹), catalase (447.57 ± 12.39 vs 530.90 ± 8.61) and malondialdehyde (23.41 ± 2.51 vs 14.14 ± 1.07 mU mL⁻¹).

Molecular docking revealed predicted energies of binding -8.0, -7.1 and -9.2 kcal mol⁻¹ between curcumin and Keap1, 15-LOX and XO, respectively, with the strongest predicted interaction observed with XO. These molecular interaction suggest that curcumin may potentially modulate proteins involved in antioxidant responses.

Overall, dietary supplementation with PAN at 0.5g.kg⁻¹ improved feed efficiency and antioxidant status in Nile tilapia under rearing conditions. The complementary In silico study provides mechanistic hypothesis linking curcumin with oxidative stress regulation and support further investigation of curcumin mediated pathways underlying the physiological effect of PAN.

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Table 1: Growth performance and feed utilization of Nile tilapia (*O. niloticus*) under rearing condition

	Control	PAN
Initial Weight (g. fish⁻¹)	74.86 ± 1.27	73.59 ± 1.10
Final Weight (g. fish⁻¹)	186.10 ± 2.29	190.72 ± 4.09
Weight Gain (g. fish⁻¹)	111.24 ± 3.2	117.13 ± 5.21
SGR (% day⁻¹)	1.15 ± 0.02	1.19 ± 0.02
FCR	1.46 ± 0.03 ^a	1.24 ± 0.03 ^b
PER	2.25 ± 0.05 ^a	2.69 ± 0.08 ^b

Values are expressed as mean ± SE (n = 3). Different superscript letters within the same row indicate significant differences among dietary treatments ($p < 0.05$).

THE EFFECT OF MALE CHANGE ON PARENTAGE CONTRIBUTION, EGG PRODUCTION AND QUALITY IN GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

Gilthead seabream (*Sparus aurata*) is the most important fin fish species in Mediterranean aquaculture, with global production of 341,000 tons in 2023. Gilthead seabream broodstocks are well adapted in aquaculture and spawn volitionally for a prolonged period of time (December to May), with very high fecundity (2 million eggs kg⁻¹ body weight) and fertilization success (Papadaki et al., 2024). Selective breeding programs have been implemented in many European countries, using family or mass selection (Janssen et al., 2017). However, the effectiveness of mass selection programs has been limiting, due to the poor male parental contribution to each day's spawning event. The objective of the present research was to examine the potential of a management method to increase male parentage contribution in gilthead seabream spawning, thus resulting in larger numbers of families and more even progeny distribution. Therefore, we abruptly replaced the males with new individuals in replicate populations and examined the effect i) on egg production and quality and later ii) on male parental contribution in the mass-spawning events immediately after new male introduction.

Materials and methods

Two broodstocks (1:4 male:female, 20 individuals), were monitored during the spawning season of 2025-2026. On 27 January 2026 (day 0), all males were replaced with new, 2-year-old spermiating males. Daily relative fecundity and fertilization success were recorded. Egg quality was evaluated at key developmental stages (24-h embryo survival, hatching, and 5-day larval survival) using 96-well plate assays. Sperm quality, including total, progressive, and rapid motility and velocity parameters, were assessed using computer assisted sperm analysis (CASA) in old and new males. Statistical comparisons between periods (before vs after replacement) were conducted using Welch's t-tests. DNA was also extracted from the breeders and from 100 eggs per spawn, 3 days before and 3 days after male exchange, from each broodstock. All samples were genotyped at 12 microsatellite loci using multiplex PCR. Fragment analysis was performed on an automated capillary sequencer, and allele scoring enabled assignment of offspring to parental genotypes. Pre- and post-replacement periods were compared using Welch's t-tests.

Results and discussion

Following male replacement, daily relative fecundity showed no significant change ($p = 0.637$). Fertilization exhibited a decreasing trend ($p = 0.091$), while the other quality parameters remained high, and no consistent differences between the periods. Sperm velocity parameters differed between the old and new males and significant reductions were observed in motility-related parameters. The percentage of motile, progressive and rapid sperm cells was markedly lower in the new males ($p < 0.001$ for all), while straightness (STR) of the trajectory remained unchanged ($p = 0.933$).

The data indicate that abrupt exchange of males during the reproductive season does not affect egg production and quality, even if the sperm motility characteristics of the new males added indicated a lower quality. Our analyses are ongoing and the final results on male parental contribution in the mass-spawning events immediately after new male introduction will be presented in the meeting.

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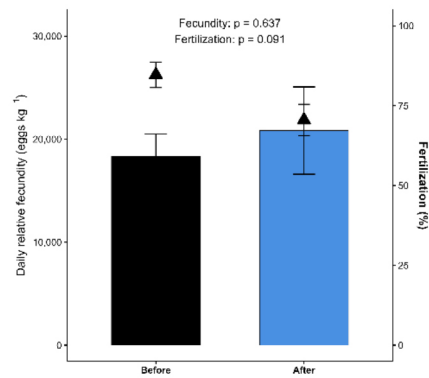


Figure 1. Mean $\pm$ SEM daily relative fecundity (bars, eggs kg⁻¹) and fertilization success (triangles, %) in two replicate populations of gilthead seabream, 3 days before and after male replacement

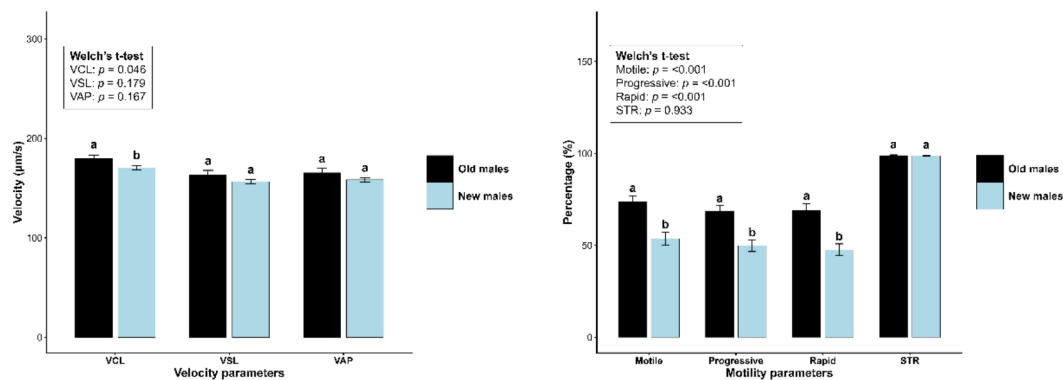


Figure 2. Mean $\pm$ SEM curvilinear (VCL, $\mu\text{m sec}^{-1}$), straight line (VSL, $\mu\text{m sec}^{-1}$) and average path velocity (VAP, $\mu\text{m sec}^{-1}$) for gilthead seabream already in a broodstock vs the new younger males used to replace them (left). Percentage (%) of motile, progressive and rapid szoa, and straightness of the trajectory (STR) (right). Letter superscripts indicate differences between groups in each parameter (Welch's t-Test, $P < 0.005$).

Acknowledgements

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EVALUATION OF AROMATASE INHIBITOR LETROZOLE TO INDUCE MASCULINISATION IN ADULT DUSKY GROUPEL (*Epinephelus marginatus*)

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Introduction

The dusky grouper (*Epinephelus marginatus*) is a cosmopolitan species of importance for fisheries, aquaculture, and ecological interest. Like many other species in this family, dusky groupers are monandric protogynous hermaphrodites, characterized by slow-growth, late maturation and long lifespan, with natural sex inversion from female to male occurring around 80 cm total length and above 7 years old (Condini et al., 2018). These biological characteristics, combined with intense fishing pressure experienced since the last century and increasing habitat destruction, led to this species being classified as Vulnerable on the IUCN Red List in 2016. Considering its economic value, especially in Asian and Mediterranean countries, this species has long been of interest for capture-based aquaculture. However, given the high feed conversion ratio and the conservation status of wild stocks, this practice seems to be unsustainable. Furthermore, sudden mass mortality events, such as those caused by Betanodavirus in 2023 and 2024 in the Mediterranean populations (Kersting et al., 2024), have highlighted the urgent need to establish aquaculture breeding programs, allowing therefore to satisfy the market demand without impacting on the wild population, as well as to provide readily available stock for reintroduction in case of worsening conservation status. The main limiting factor in dusky grouper breeding is currently the difficulty in inducing sex inversion to males, which results in lack of sperm for fertilization. To address this issue, aromatase inhibitors have already been used in other congeners with successful and permanent results. However, in dusky groupers they have rarely been tested alone, and results were often limited to very low milt production or temporary inversion. The objective of this research is, therefore, to test letrozole, a non-steroidal aromatase inhibitor, in order to evaluate its effectiveness in inducing masculinization in individuals who are already within the natural range of sex inversion.

Materials and methods

Fifteen dusky groupers of average weight 6.3 ± 0.9 kg (mean $\pm$ SD) and around twenty-five years old were maintained in two rectangular tanks of 5 m³ in the broodstock facility of Aqualabs at the Institute of Marine Biology, Biotechnology and Aquaculture, in Crete. Starting on 11th of March and every 28 days, letrozole (InvivoChem, China) has been injected in fish dorsal muscle in two different experimental doses, both diluted in 50 μ l fish oil kg⁻¹ of fish. Fish were randomly divided in three groups receiving respectively effective dose of 1.07 ± 0.02 mg kg⁻¹ letrozole (n=5), 10.45 ± 0.18 mg kg⁻¹ letrozole (n=5) and fish oil only as control (n=5). Fish were reared together in similar stocking density. On day 0, before starting the treatment, no sperm was found in the groupers as well as no gonadal biopsies were possible to be obtained with the catheter, probably due to underdeveloped gonads, as fish were outside their reproductive season. On the same day of injection and every 14 days, the groupers were checked for sperm production and approximately 1.5 ml of blood was collected with heparinized syringes. Masculinization and spermiation will be examined every 14 days, by applying gentle abdominal pressure and collecting sperm. Sperm quality will be analyzed with Computer Assisted Sperm Analysis (CASA) for sperm density, spermatozoa motility (%), progressive motile spermatozoa (%), rapid motile spermatozoa (%), straight-line velocity (VSL, μ m sec⁻¹), curvilinear velocity (VCL, μ m sec⁻¹), average path velocity (VAP, μ m sec⁻¹) and straightness (STR, %). Plasma levels of 17- β Estradiol (E₂), Testosterone (T), 11-Ketotestosterone (11-KT) and 17 α ,20 β -Dihydroxy-4-pregnen-3-one (17 α ,20 β -P) will be quantified using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS).



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Results and discussion

The experiment is currently ongoing and the results are expected to be obtained around mid of June, at the end of letrozole treatment and in correspondence of the beginning of the natural spawning season. An update of this abstract will follow at the end of the experimental trial. Considering the inhibiting action of letrozole on the enzyme responsible for testosterone aromatisation into 17- β estradiol (CYP19A1), in letrozole-injected groupers the plasma concentration of 17- β estradiol is expected to decrease compared to control fish, whereas testosterone and 11-ketotestosterone should increase. The maturation inducing steroid 17 α ,20 β -dihydroxy-4-pregnen-3-one, instead, is likely to remain constant or slightly increase in treated groupers, therefore stimulating spermiation and sperm motility. Furthermore, hypothesizing a complete masculinization of groupers, a second experiment will be later conducted to evaluate their effective capability to fertilize eggs under natural spawning conditions.

Acknowledgements

This study is funded by the internal project ELEBOOST-Fakr awarded to IF.

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METABOLIC RESPONSES OF JUVENILE PACU TO COMBINED THERMAL AND MICROPLASTIC STRESS: IMPLICATIONS FOR AQUACULTURE UNDER GLOBAL CHANGES

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Introduction

Understanding how multiple environmental stressors simultaneously affect the physiology and metabolism of farmed fish remains limited, representing a growing challenge for aquaculture under climate change and increasing production demands. Temperature fluctuations, particularly extreme cold and heat events, can alter energy demands and metabolic resource allocation required to maintain homeostasis, while microplastics (MPs), which are emerging aquatic contaminants, may interact with thermal stress and potentially exacerbate metabolic disturbances. In this context, this study investigated the combined effects of MPs and temperature on the metabolic responses of juvenile pacu (*Piaractus mesopotamicus*), a native species of relevance to Brazilian aquaculture. Fish were exposed for 96 h to two MP concentrations (0 and 20 µg L⁻¹) at three temperatures (16, 28, and 34 °C), using 24 aquaria with four replicates per treatment and two fish per aquarium. At the end of the exposure period, plasma glucose and serum triglyceride and cholesterol levels were assessed as indicators of metabolic alterations.

Results and Discussion

After 96 h, fish maintained at 28 °C exhibited the lowest plasma glucose levels, whereas higher glucose concentrations were observed at 16 and 34 °C, suggesting increased energy demand to maintain homeostasis under suboptimal thermal conditions. This pattern is consistent with previous findings in pacu showing that cold exposure promotes the mobilization of energy substrates and increases blood glucose levels (Ale et al., 2021). MP exposure further increased glucose levels at 16 °C compared with non-exposed fish at the same temperature, suggesting a potential interaction between thermal stress and MP exposure. Triglyceride levels were also lower at 28 °C, regardless of MP exposure, whereas the highest concentrations were observed in fish exposed to MPs at 16 °C, indicating alterations in lipid metabolism under combined stress conditions. Conversely, cholesterol levels were higher at 34 °C in MP-exposed fish, suggesting that MPs may modify lipid metabolism in a temperature-dependent manner. Overall, the observed changes in glucose, triglycerides, and cholesterol are consistent with disturbances in energy homeostasis reported in fish exposed to suboptimal temperatures and MPs (Ale et al., 2021; Ghosh, 2025). These findings demonstrate that extreme temperatures alter metabolic homeostasis in pacu and may modulate the physiological effects of MP exposure, resulting in temperature-dependent metabolic responses. Changes in glucose, triglycerides, and cholesterol indicate adjustments in carbohydrate and lipid metabolism under adverse environmental conditions. These results highlight the importance of considering multiple interacting stressors when assessing the adaptive capacity and resilience of farmed fish, particularly in the context of climate change and the increasing frequency of extreme thermal events in aquaculture systems.

Acknowledgments

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MATCHING SALMONID SKYN DYNAMICS: PROXY MODELS FOR HEALTH AND NUTRITIONAL SCREENING

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Introduction

Bacteria infections, parasite infestations and environmental stressors present severe health challenges in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) aquaculture with consequences in the health and defensive capacity of the skin mucosa. While both salmonid species share close phylogenetic traits, determining whether their skin tissues share equivalent sensitivity and responsiveness to physiological insults is crucial for understanding mucosal defense mechanisms across species. Using a controlled ex vivo skin system, this study compared the cutaneous transcriptional responsiveness of Atlantic salmon and rainbow trout exposed to identical oxidative and immune stimuli. Establishing whether both species exhibit matching response patterns under standardized conditions is essential for future cross-species applications aimed at evaluating novel nutritional strategies while complying with 3Rs policies (Replacement, Reduction, Refinement) to minimize live animal testing.

Material and methods

Skin explants were collected from anesthetized Atlantic salmon and rainbow trout and incubated for 6 h or 24 h under two controlled challenges: oxidative stress induced by hydrogen peroxide (H₂O₂) and an immune challenge simulated by two homogenates of sea lice with different processing. Cutaneous responsiveness was evaluated via real-time quantitative PCR (qPCR) targeting a panel of biomarkers for immune response (e.g. *il1b*, *il8*, *tnfa*), antioxidant defense (e.g. *cat*, *gpx1*, *nrf2*), and epithelial integrity (e.g. *tjp1*, *oc1*, *agr*). Relative gene expression dynamics were directly compared between species across all conditions.

Results and discussion

Under standardized conditions, the skin mucosa of both Atlantic salmon and rainbow trout exhibited matching transcriptional response patterns across all tested challenges. Exposure to sea lice homogenates induced a strong pro-inflammatory response (*il1b*, *il8*, *tnfa*) in both species, showing significantly greater expression at 24 h compared to the 6 h timepoint. Furthermore, biomarkers targeting antioxidant defense and epithelial integrity displayed parallel temporal modulation, with no major species-specific divergence. These uniform expression trends demonstrate a shared mucosal homeostatic strategy and equivalent biological reactivity in both salmonids when subjected to homologous stressors.

Conclusion

Atlantic salmon and rainbow trout skin display equivalent transcriptional responsiveness to oxidative and parasitic insults. Confirming that both species share identical response patterns under controlled conditions validates using one species as a direct proxy for the other. This insight provides a reliable foundation for future applications evaluating the functional impacts of nutritional and health-management strategies in skin responses, advancing formulation pipelines while fully adhering to 3Rs policies.

Acknowledgment

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OXYTETRACYCLINE STRESS RESPONSES IN AQUACULTURE *Levilactobacillus brevis* and *Lactococcus petauri*: A GENOMIC AND PROTEOMIC INSIGHT

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Introduction

Antibiotic resistance in aquaculture poses a serious threat to aquatic biosecurity and environmental health. Antibiotic resistance (AMR) in aquaculture is a critical global concern for biosecurity and environmental health. Lactic acid bacteria (LAB) isolated from aquatic environments play a dual role as potential probiotics and potential reservoirs of resistance determinants. Understanding their functional diversity and adaptive responses to antibiotic stress is essential for sustainable aquaculture management.

Materials and Methods

Two multidrug-resistant LAB strains isolated from aquaculture systems in Peru—*Levilactobacillus brevis* UC6 (resistant to 11 antibiotics) and *Lactococcus petauri* UC20 (resistant to 5 antibiotics)—were selected for integrated omics characterization. Whole-genome sequencing was performed, followed by hybrid assembly and functional annotation (Bakta, METABOLIC, CARD/RGI). Quantitative label-free proteomics was conducted using data-independent acquisition (DIA-MS) with dia-PASEF technology on an Evosep One coupled to a timsTOF Pro 2 mass spectrometer. Differentially expressed proteins (DEPs) were evaluated under basal control and oxytetracycline stress conditions. Uncharacterized overexpressed DEPs were modeled in 3D using ESMFold/AlphaFold and structurally aligned via Foldseek to identify novel functional homologies.

Results

Comparative genomic profiling revealed distinct metabolic and enzymatic capabilities among the strains. While sharing identical absolute counts of carbohydrate-active enzymes (CAZymes), *L. petauri* UC20 exhibited a significantly higher CAZyme density (27.07 per 1000 CDS) due to its simplified genome compared to *L. brevis* UC6 (20.40 per 1000 CDS). Conversely, UC6 demonstrated an expanded genetic repertoire dedicated to xenobiotic biodegradation and membrane transport. Quantitative DIA-MS proteomics identified a robust, strain-specific proteomic reprogramming under oxytetracycline exposure. Structural proteomics of major uncharacterized overexpressed proteins led to the discovery of potential novel predicted multidrug efflux transporters (MFS/ABC families) and ribosomal protection candidates that show structural 3D folding homology with established resistance proteins despite low sequence identity.

Discussion

The functional genomic divergence between *L. petauri* UC20 and *L. brevis* UC6 reflects species-specific metabolic specialization, where genome simplification determines CAZyme density, while expanded transport systems drive multidrug resistance (Du et al., 2018). Under oxytetracycline stress, the marked proteomic reprogramming observed by DIA-MS demonstrates significant remodeling driven by uncharacterized pathways alongside expected mechanisms (Nielsen et al., 2014). Crucially, demonstrating that hypothetical overexpressed proteins adopt classic 3D structural folds of MFS/ABC efflux pumps and ribosomal protection factors validates the efficacy of 3D structural comparison for uncovering non-canonical resistance determinants (van Kempen et al., 2024).

Conclusion

These findings highlight the distinct metabolic adaptation strategies and proteomic responses employed by *L. brevis* and *L. petauri* under antibiotic selection pressure. The combination of quantitative DIA-MS proteomics and 3D structural fold alignment represents a powerful workflow to uncover unannotated multidrug resistance determinants in aquaculture microbiomes.

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Acknowledgments

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COMPUTER VISION FOR AUTOMATED PHENOTYPING: WEIGHT PREDICTION FOR GENOMIC EVALUATION OF GROWTH AND RESILIENCE INDICATORS (LnVar) IN YELLOWTAIL KINGFISH (*Seriola lalandi*)

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Introduction

Only a very small portion of global aquaculture production is derived from structured breeding programs. A major bottleneck in implementing aquaculture breeding programs is the difficulty of removing aquatic animals from their water environment to measure phenotypes. Over the last decade, the development of machine learning (ML) and computer vision (CV) techniques has provided non-invasive alternatives using image and video analysis to be applied in animal production systems, opening a new possibility for high-throughput phenotyping with minimal disturbance to the animals. This study uses computer vision to predict animal weight directly from images for estimating phenotypic data in Yellowtail Kingfish (*Seriola lalandi*) under commercial conditions, simplifying the repeated measurements that are required for phenotypes such as growth rate the LnVar resilience indicator. Repeated measurements are combined with genotypes to apply genomic analyses of these image-derived measurements.

Materials and Methods

A total of 1,600 PIT-tagged kingfish juveniles were randomly distributed into two treatment tanks at The Kingfish Company (Kats, the Netherlands) and monitored via monthly biometrics over five months. During each event, automated digital imaging (Dorset ID, Aalten, the Netherlands) was performed, and a subset was physically weighed for model calibration. Subsequently, a deep-learning segmentation model processed the images to extract pixel-based area and volume (Figure 1). Quadratic regression models predicted weights for the unweighed population using extracted pixel traits, subsequently allowing the calculation of the daily growth coefficient (DGC) and the LnVar resilience proxy (Mengistu et al., 2022). Genomically, 1,140 individuals were genotyped (Benchmark Genetics, Bergen, Norway). Post-quality control, 25,160 retained SNPs were used to construct a genomic relationship matrix (GRM) via Calc_grm (Calus & Vandenplas, 2016), which was utilized in ASREML to estimate variance components and heritabilities through a univariate model accounting for the treatment effect.

Table/Figure 1



Figure 1 Image processing pipeline for automated phenotyping

Table 1. Variance estimates and heritabilities of weight, growth, and resilience

Trait	N	V _A	se	V _E	se	h ² ± se
Weight 1	1100	302	50.8	473.5	26.7	0.39 (0.05)
Weight 2	1100	2514.2	403.6	3992.7	217.2	0.39 (0.05)
Weight 3	1132	9114.4	1383.1	12218.5	678.8	0.43 (0.04)
Weight 4	1097	29884.2	4480.4	35238.7	2051.2	0.46 (0.05)
Weight 5	1103	69974.1	10428.3	75019.3	4478.8	0.48 (0.05)
DGC 1-5	1063	0.248	0.04299	0.367	0.0217	0.40 (0.05)
LnVar	1140	0.0576	0.0287	1.296	0.0575	0.04 (0.02)

DGC: Daily Growth Coefficient; LnVar: Logtransformed variance if weight deviations;

(Continued on next page)

Results

By physically weighing a calibration subset of 100 fish per standard timepoint, the quadratic regression models applied to these calibration subsets yielded coefficients of determination (R^2) ranging from 0.914 to 0.977 and low Mean Absolute Percentage Error (MAPE) between 2.25% and 5.01%, successfully predicting the weights of the remaining unweighed population (up to 770 individuals per treatment per timepoint). Over the entire experiment, a total of 1,494 manual scale measurements estimated 6,299 individual weights. Heritabilities (h^2) for predicted body weight progressively increased from 0.39 ± 0.05 at early stages (WT 1) to 0.48 ± 0.05 at harvest (WT 5). Growth traits demonstrated similarly robust heritabilities, with 0.40 ± 0.05 for DGC, whereas resilience (LnVar) exhibited a low heritability of 0.04 ± 0.02 (Table 1).

Discussion

The computer vision approach utilized in this study successfully predicted the entire population-level weights by weighting only $17\% \pm 7.36\%$ of the sampled animals per timepoint, providing a significantly faster and reliable procedure. Regarding the genomic results, while overall growth measurements exhibited moderate heritabilities of approximately 0.4, the LnVar resilience index demonstrated near-zero heritability, rendering its evaluation suggestive at best. Study of growth variation and resilience remains of significant interest, which strictly requires frequent longitudinal phenotyping. The integration of computer vision combined with artificial intelligence in aquaculture provides a framework for rapid and precise phenotyping as demonstrated in this study. Nevertheless, to meaningfully improve animal welfare and eliminate handling-induced stress, future technologies must evolve to capture images without sedatives and, preferably, without any physical handling of the fish. As these computer vision systems advance toward fully non-invasive, in-water monitoring, they will become critical for evaluating complex resilience traits under commercial conditions.

Acknowledgment

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INITIAL EVALUATION OF COPEPODS AS FIRST PREY IN THE EARLY LARVAL FEEDING OF STRIPED RED MULLET *Mullus surmuletus*

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Introduction

The striped red mullet (*Mullus surmuletus* Linnaeus, 1758) is a promising candidate for diversification in marine aquaculture, yet its larval rearing remains poorly developed. Early larvae are extremely small and sensitive, requiring prey of adequate size and mobility to initiate exogenous feeding (Yúfera & Darias, 2007). Copepods are considered high-quality live prey due to their nutritional profile and swimming behaviour, which can enhance feeding responses in delicate larvae (Støttrup, 2000). The main objective of the SALROC research project, funded by the Xunta de Galicia, is to contribute to the diversification of marine fish farming in Europe by optimizing farming techniques for *M. surmuletus* (Fernández *et al.*, 2025 a, b; 2026 a, b) and actually is being conducted at CIMA (Centro de Investigacións Mariñas de Galicia), CETGA (Centro Tecnológico del Clúster de la Acuicultura) and CSIC (Centro Superior de Investigaciones Científicas). Within the SALROC project, we evaluated the performance of *M. surmuletus* larvae fed exclusively with copepods during a larval trial, aiming to identify bottlenecks in survival, feeding onset and early development.

Materials and Methods

A batch of ~8000 eggs from CETGA broodstock was incubated at 15 °C in 60 L tanks (open circuit). Upon hatching, ~2600 viable larvae (hatching rate 32,5%) were transferred to two 140 L tanks at 19 °C. Copepods (*Acartia tonsa*, *Tisbe* spp.) enriched with *Rhodomonas lens* were sieved at 50 µm and supplied at ~10 ind·mL⁻¹. Larval development, survival, pigmentation, mouth opening and digestive tract condition were monitored through stereomicroscopy and high-resolution imaging.

Results

Figure 1 (a, b, c, d) shows some images of the development of *M. surmuletus* eggs and larvae obtained in this study.

A video of a developing *M. surmuletus* embryo, captured using Light-Sheet Fluorescence Microscopy (LSFM), is available at the following link:

<https://youtube.com/shorts/loSwdm6V0Os>

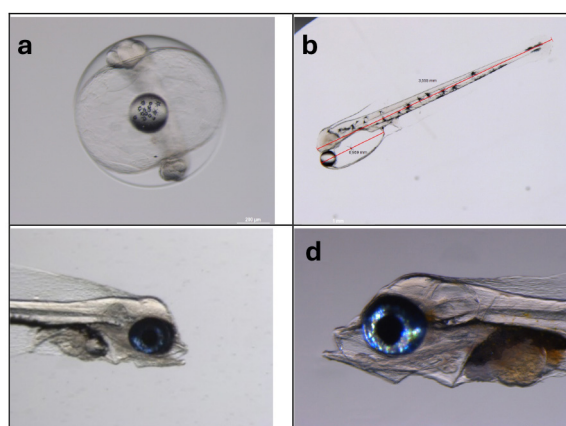


Figure 1. Different developmental stages of *Mullus surmuletus*. **1a:** Egg 2 DPF. **1b:** Larva 3 DPH. **1c:** Larva 9 DPH. **1d:** Larva 28 DPH. DPF: Days post fertilization. DPH: Days post hatching.

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Larval quality at hatching was heterogeneous, with frequent axial deformities. Two mortality peaks occurred: at 4 DPH (coinciding with mouth opening and yolk exhaustion) and at 10 DPH, reducing the population to ~200 larvae per tank. Although most larvae showed no clear feeding behaviour, some individuals displayed darkened stomach regions at 7–11 DPH, compatible with partial ingestion of copepods. Growth remained arrested around 4 mm total length for the majority of larvae. However, one individual reached 10.02 mm at 28 DPH, indicating sustained ingestion and effective assimilation of copepod prey.

Discussion

The study showed that the onset of exogenous feeding is the main constraint in early *M.surmuletus* rearing. Although a few larvae exhibited signs of partial copepod ingestion, most individuals failed to initiate sustained feeding and remained growth-arrested at ~4 mm. Mortality peaks at 4 and 10 DPH highlight the critical nature of the first-feeding window and suggest that copepods may not be fully adequate as initial prey.

Although copepods might initially be considered a suitable option for feeding small-mouthed fish larvae, such as striped red mullet, due to the reduced size of their nauplii and their generally favorable nutritional profile, typically rich in polyunsaturated fatty acids (PUFAs), this trial suggests that exclusive early feeding with copepods is not feasible. Copepod nauplii may be too fast to be efficiently captured by early larvae, and/or larvae may not digest them properly during the initial stages. These observations indicate that the first feeding of striped red mullet larvae should be complemented with smaller and less mobile prey, either by using copepod species with less motile nauplii or enriched rotifers of the smallest possible size, such as *Brachionus rotundiformis*.

At more advanced developmental stages, newly hatched *Artemia*, a widely established intermediate feed in marine larviculture due to its appropriate size, ease of handling and well-documented performance (Dhont *et al.*, 2013), can be incorporated into the diet. Adult copepods could then be introduced once larvae have greater robustness and feeding ability, as they are known to support superior growth in later stages (Støttrup, 2000).

Acknowledgment

This work was funded by the Consellería do Mar (Xunta de Galicia), through project “Diversificación de especies na piscicultura mariña galega: cultivo do salmonete de roca (*Mullus surmuletus*)”, CIMA 24/01 (SALROC).

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POTENTIAL ROLE OF MICROBIOTA IN DISEASE RESISTANCE OF *Magallana gigas* OYSTERS

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Introduction

The Pacific oyster, *Magallana gigas*, is the main oyster’s species cultivated worldwide and one of the most important species in aquaculture. However, its production has begun to decrease due to degradation of the environment and to infectious disease outbreaks, more specifically by two pathogens: the virus OsHV-1 and the bacteria *Vibrio aestuarianus* (Azema et al. 2016).

Although selective breeding (SB) is a great tool to improve a trait of interest, in this case, the resistance to pathogens, some other potential factors such as the microbiome profile of the oyster can have an important role in the development and propagation of the disease.

In this sense, three families of oysters belonging to a breeding program and three unselected ones were infected with a highly pathogenic strain of *V. aestuarianus* and the microbiome content of the oyster was analyzed after 30 days of infection to determine its potential effect in the resistance/susceptibility.

Material and Methods

Six families (100 oysters/family, 32 months old), were used, , three of them were selected for their higher resistance to both pathogens and the three others were unselected. All oysters were anesthetized, and half of them (50 per family) were injected using 100 μ l (1.8×10^7 CFU) of *V. aestuarianus* suspension into the adductor muscle (injected condition), while the other half were injected with 100 μ L of sterile artificial sea water (control condition). Oysters were then transferred into 50L tanks at $19 \pm 1^{\circ}\text{C}$, in a close system with 100% water renewal every two days. Mortality was recorded daily for 28 days.

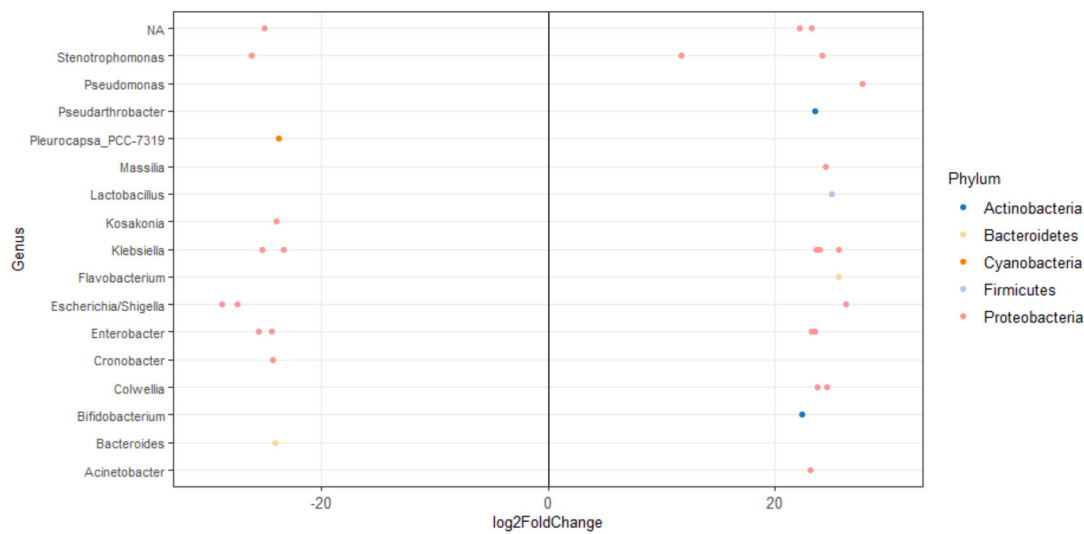


Figure 1. Genus significantly different between the unselected vs selected group at 30 days post injection.

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Bacterial DNA from gill was extracted from six live oysters per family and timepoint (24 hours and 30 days post injection). After extraction, the hypervariable region V3-V4 of the 16S was sequenced in an Illumina platform at BMKGENE (Germany). Samples were analyzed using the DADA2 (v1.16) pipeline in R studio. The identification of differentially abundant taxa between experimental groups was performed using the DESeq2 package according to Semedo and Song (2020).

Results

Mortality in selected families ranged from 29 to 36% while in unselected ones ranged from 51 to 84%. Alpha diversity showed a similar pattern among families with no differences between timepoints. This analysis showed that, in general, *V. aestuarianus* infection do not alter the bacterial community provoking major changes in richness (Chao index) or overall diversity (Shannon index). Although some differences were expected, at least between timepoints, it seems that neither the timepoint, neither the infection had a major effect on the microbial composition between families. All families presented a similar microbiota composition dominated by Gammaproteobacteria, Bacteroidia, Alphaproteobacteria and Actinobacteria regarding the class composition.

But, although no differences were found regarding the major groups, when we take a closer look at genus composition, some differences appeared. At 24 hours post injection the bacterial community was more homogeneous but, the image changed completely after 30 days post injection, with some major differences were evident between the selected and unselected oysters. For the unselected oyster, a drift to colonization of *Enterobacter*, *Cronobacter*, *Scherichia/Shigella*, *Streptococcus*, *Tenacibaculum* and *Sulfitobacter* was evidenced (Fig. 1). On the other hand, *Colwellia*, *Rothia*, *Pseudomonas*, *Pseudarthrobacter*, *Paenibacillus*, *Massilia*, *Marinomonas* and *Marifilum* became more present in the selected group (Fig 1). Although neither Chao or Shannon index showed differences between groups, it seems that, selected oysters present a more diverse and complex taxa with also more genus belonged to beneficial bacteria.

Conclusion

Some bacterial groups were directly linked to resistance or susceptibility to the *V. aestuarianus*. Bacteria from genus *Colwellia*, *Marinomonas* and *Tenacibaculum* were more present in the selected group while *Arcobacter*, *Vibrio*, *Enterobacter* and *Streptococcus* were more present in the unselected group.

It is possible to conclude that SB programs are an important key to enhance the resistance of *M. gigas*, but also the microbiome could have a potential role to improve the performance of animals.

Acknowledgments

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OPTIMIZATION OF BIOFLOC SYSTEMS FOR SUPER-INTENSIVE *Penaeus vannamei* CULTURE IN EUROPEAN CONTEXT

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Introduction

The production of *Penaeus vannamei* represents the main sector of global aquaculture; however, its intensification raises sustainability challenges, particularly related to fishmeal use and nitrogen waste management. Biofloc technology (BFT) has emerged as an alternative based on nutrient recycling into microbial biomass, improving water quality and providing a complementary nutritional source. However, its performance depends on multiple interrelated factors that have traditionally been studied in isolation. This study adopts an integrated approach, evaluating system optimization, nutritional efficiency, microbiota, and product quality to support more sustainable shrimp production.

Material & Methods

A series of experiments were conducted under super-intensive conditions in BFT systems. The following aspects were evaluated: (i) the effect of carbon sources (molasses, yeast, and polymers) and C:N ratios (10:1, 18:1, 26:1) on water quality, microbial dynamics, and growth; (ii) nutritional efficiency through different protein levels (30–46%) and feeding regimes (70–100%), including stable isotope analysis to estimate biofloc contribution; (iii) fishmeal replacement using isoproteic and isolipidic diets with alternative protein sources; (iv) characterization of biofloc and gut microbiota through 16S rRNA gene sequencing; and (v) preservative-free post-harvest quality, assessing slaughter methods and their effects on microbial load, spoilage, and sensory properties during refrigerated storage.

Results & Discussion

Results demonstrated that biofloc system performance is strongly influenced by carbon source and C:N ratio, affecting water quality, microbiota, and shrimp growth. Molasses, particularly at low to intermediate C:N ratios, emerged as the most suitable strategy, promoting a balanced and functional microbial community. This is likely due to greater carbon availability stimulating heterotrophic bacteria compared to slow-release sources such as polymers, despite better nitrogen control and higher diversity (Luo et al., 2019). In contrast, yeast treatment showed higher phosphate accumulation and a more dominated bacterial composition, limiting their productive advantage (Emerenciano et al., 2017). These results indicate that heterotrophic activity is more relevant than diversity per se (Samocha et al., 2020). Stable isotope analysis confirmed that biofloc contributes ~40% to shrimp nutrition allowing a 30% reduction in feeding rate without affecting growth and saving €0.95/kg biomass, confirming overestimated requirement by conventional feeding tables (Lara et al., 2017). Dietary protein was optimized to 38% in juveniles and 34% in grow-out, consistent with reduced requirements as shrimp grow (Henriques et al., 2021). Up to 92% FM replacement using alternative protein sources was feasible without compromising performance, confirming the compensatory role of biofloc and supporting reduced FM use (Chen et al., 2024). Diet also influenced product quality, with mixed diets providing the best balance between sensory attributes and reduced melanosis. Microbiota analysis revealed clear differentiation between biofloc and gut communities, mainly driven by system maturation (Kim et al., 2022) and shrimp growth (Gainza et al., 2018), rather than diet. Finally, slaughter methods affected preservative-free post-harvest quality, the most suitable method consist of a 5-minute immersion in chilled seawater with 50 ppm sodium hypochlorite followed by thermal shock in ice slurry. Overall, these results demonstrate that BFT can be optimized through an integrated approach that improves efficiency, reduces feeding costs, enhances sustainability, and improves product quality.

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Acknowledgment

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OPTIMIZING AEROBIC AND ANOXIC DENITRIFICATION IN *Peneus vannamei* UNDER BIOFLOC PRODUCTION SYSTEMS

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Introduction

Biofloc technology (BFT) enables intensive aquaculture production with low water exchange, but this can lead to the accumulation of nitrogen compounds, particularly nitrate. Nitrate buildup may affect water quality, animal performance, and system sustainability, and may also pose an environmental risk if untreated water is discharged. In BFT systems, nitrate removal has traditionally been linked to denitrification under anoxic or anaerobic conditions. However, recent evidence suggests that denitrification may also occur under aerobic conditions, highlighting the functional complexity of biofloc microbial communities.

Material and methods

Two trials were carried out to evaluate aerobic and anoxic denitrification in *P. vannamei* production system. First, a 48 h assay was conducted to optimize nitrate removal in external bioreactors using biofloc with approximately 300 mg/L total suspended solids (TSS) and water with a high nitrate concentration. Anoxic denitrification was performed by adding molasses to adjust the C:N ratio to 3:1, and removing aeration. While, aerobic denitrification was performed under controlled oxygenated conditions (>4 mg/L) and adjusting the C:N ratio to 20:1 with a carbon source composed of a mix of di-, poly- and monosaccharides (Maltose 53%, corn starch 24% and glucose 2%). Microbial populations were analyzed by qPCR to assess the functional genes associated with nitrification and denitrification under each condition. In the second trial, the denitrification strategies were applied for 4 weeks in shrimp production tanks. Once per week, between 15-20% of the tank water was subjected to a 48 h denitrification period under either anoxic or aerobic conditions and then reintroduced into the production system. In both trials, water quality was monitored through dissolved oxygen, temperature, salinity, pH, alkalinity, ammonium, nitrite, and nitrate. The pH was maintained above 8 by adding 1 M NaOH, and each denitrification period was considered complete when nitrate concentrations approached 0 mg/L.

Results and discussion

The results showed that both strategies reduced nitrate in biofloc systems, although through different dynamics. In the optimization trial, anoxic denitrification achieved the highest nitrate removal efficiency, close to 95.7%, but was accompanied by transient nitrite and ammonium accumulation and increased alkalinity after pH correction, indicating greater operational instability. In contrast, the aerobic pathway showed a more gradual and lower nitrate reduction, approximately 77.2%, but maintained greater water quality stability. The increase in TSS under this condition suggests a relevant contribution of heterotrophic assimilation, consistent with carbon supplemented biofloc systems (Ebeling et al., 2006; Avnimelech, 2009). qPCR analysis supported these differences: under anoxic conditions, the upward trend in *narG*, together with the low abundance of downstream denitrification genes, were consistent with transient nitrite accumulation; whereas, under aerobic conditions, the higher abundance of *narG*, *nirS*, and *nirK*, together with the increase in AOB and *Nitrospira* trend, suggests the coexistence of nitrification, heterotrophic assimilation, and denitrification in anoxic microenvironments within the flocs (Deng et al., 2020; Bhattacharya and Mazumder, 2021).

In the *in vivo* trials with *P. vannamei*, the weekly use of external reactors helped control nitrate accumulation. The anoxic pathway produced a more marked reduction of nitrate in the culture tanks compared with the control, reaching concentrations close to 0 mg/L in the reactors. However, it required a longer treatment time (96 h), to avoid risks associated with nitrite, ammonium, and low dissolved oxygen. In contrast, the aerobic pathway was more stable, but its impact on nitrate accumulation in the culture tanks was limited, likely because continuous nitrate production from feeding, excretion, and nitrification partially exceeded the treatment removal capacity. This agrees with the preliminary trial and with the higher relative abundance of nitrifying bacteria observed under aerobic conditions (Kim et al., 2022; Han et al., 2024). Additionally, both strategies were viable and did not compromise shrimp growth or survival, in agreement with previous

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studies using denitrified water in biofloc systems (Brandão et al., 2024). Therefore, despite requiring stricter management, anoxic denitrification appears to be the most promising strategy for effectively controlling nitrate accumulation in biofloc systems; while the aerobic pathway provides greater operational stability, but further optimization is needed to achieve comparable results under culture conditions.

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THE INFLUENCE OF PRODUCTION ENVIRONMENTS ON CARDIAC HEALTH AND FUNCTIONS IN ATLANTIC SALMON

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Introduction

Recirculating aquaculture systems (RAS) have emerged as a promising alternative to the traditional flow-through (FT) system for land-based rearing of Atlantic salmon, offering advantages regarding water usage, biosecurity and control of environmental parameters. However, RAS is often associated with higher concentrations of metabolites and residues in the system, which lowers water quality and potentially increases the vulnerability of fish to multifactorial stressors encountered later in the marine phase. Climate change has become a significant concern in sea-based production, as it can intensify adverse environmental conditions. Elevated water temperatures, reduced oxygen levels, and exposure to jellyfish blooms have been shown to negatively affect fish growth and welfare, yet limited research has focused on the combined effects of multiple stressors. The study examines how production environments, particularly RAS and FT, influence the cardiac health and function of Atlantic salmon, and whether these systems affect susceptibility to climate-related stressors during the marine phase. The findings provide insight for developing climate adaptation strategies and reducing fish morbidity and mortality in aquaculture.

Materials and methods

For the Main Trial, Atlantic salmon parr were divided to be reared in FT or RAS. Fish were subjected to 5 weeks of continuous illumination and a gradual salinity increase to induce smoltification, with heart samples being collected 1 day and 14 days after. Seawater transfer was simulated, and the fish were divided into Control and Test groups. The Test groups were subjected to a stress test that included a gradual increase in water temperature from 12°C to 17°C (1°C per day), followed by a decrease in dissolved oxygen (DO) to 60% for 24 hours, and, lastly, exposure to macerated jellyfish. Hearts were sampled 1 day and 5 days post-exposure. Additionally, 9 fish from each group were selected for a 10-minute electrocardiogram (ECG) assessment, during which hypoxic bradycardia was induced by ceasing artificial gill ventilation in anaesthetised fish. Heart samples were sectioned into atrium, bulbus arteriosus, and ventricle, and differences in the expression of genes related to inflammation, thermal stress, angiogenesis, cardiac growth/remodelling, and hypoxia were analysed by RT-qPCR. Histology and immunohistochemistry were also conducted.

Results

The gene expression profile of the Main Trial showed statistically significant differences between the FT and RAS production environments in atrium and bulbus arteriosus samples, specifically in *ctgf* and *cyr61*, both markers of angiogenesis, with higher relative expression in the RAS groups. In contrast, ventricle samples showed no significant upregulations related to the production systems. Multiple significant differences between the two sampling points were identified across all heart compartments in cardiac growth and remodelling-related genes, including *colla* and *anp*, as well as the previously mentioned angiogenesis markers. In the climate change stress challenge, only one statistically significant upregulation in the RAS group was noted in *heat shock protein 70*, a thermal stress-related gene, within bulbus arteriosus samples. Atrium and ventricle samples showed similar expression patterns in both the FT and RAS environments. Significant differences between sampling points or between the Control and Test groups were frequent across all heart compartments for angiogenesis, thermal stress, and cardiac growth/remodelling markers. The relative expression patterns were maintained throughout the three heart regions, especially in genes associated with inflammation, thermal stress and hypoxia. Results from the ECG assessment revealed no differences between the FT and RAS groups. Induced hypoxic bradycardia was also comparable between FT and RAS, however, in the Test group, the bradycardic response was attenuated one day following jellyfish exposure, with signs of recovery evident 5 days post-exposure. Histology and immunohistochemistry samples are currently being analysed.

Discussion

Results from the gene expression analysis showed only minor differences between the FT and RAS production environments, with similar trends in relative expression observed across all three heart compartments and in both trials. This consistency was particularly evident for genes related to inflammation, thermal stress, cardiac growth and remodelling, and hypoxia. These preliminary findings suggest that land-based RAS, under the conditions in the experimental set-up, does not compromise cardiac health in Atlantic salmon, nor does it increase susceptibility to climate-related stressors encountered during the marine phase, compared with FT rearing.

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EXTRACTS OF THE HALOPHYTE SALICORNIA MODULATE THE FUNCTIONS OF ATLANTIC SALMON HEAD KIDNEY ADHERENT LEUKOCYTES AND GILL EPITHELIAL CELLS

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Introduction

Novel functional feed-additives capable of improving fish health have gained attention in recent years. An increasing array of plant-derived compounds are being screened for their potential health safe-guarding characteristics. Recently halophytes, such as *Salicornia ramosissima*, are gaining traction as aquafeed supplements due to their potential immunomodulatory effects. Employing cells derived from Atlantic salmon (*Salmo salar*), the effects of several *S. ramosissima* extracts were assessed by conducting both functional and transcriptomic studies.

Materials and Methods

Head kidney primary cells were harvested from healthy Atlantic salmon, and adherent leukocytes (ASAL) were obtained following previously established protocols¹. In addition, we employed the gill epithelial cell line ASG-10 that has been widely employed in *in vitro* studies². These cells were exposed to different types of *S. ramosissima* extracts (labelled as EXT3, XAD and FERM), derived from distinct steps of the extraction cascade. The MTT (for ASAL) assay and propidium iodide staining-based analysis employing imaging flow cytometry (IFC; for ASG-10) determined the concentrations that are not toxic to the cell types. Suitable concentrations were then selected for the cell exposure studies.

Cells were exposed to each extract for either 3 or 24 h, followed by a challenge with inactivated pathogenic bacteria *Tenacibaculum maritimum*. Phagocytosis and reactive oxygen species (ROS) production were quantified employing IFC protocols¹. Furthermore, a wound healing assay was performed on gill epithelial cells using an automated scratch system followed by live cell imaging. In parallel, a snapshot of the changes in the transcriptome of the two cell types was captured to reveal their molecular responses.

Results and Discussion

In the case of adherent leukocytes, only XAD elicited significant responses - an enhancement of phagocytosis and ROS production after 24 h exposure. These responses occurred after the transcriptional reprogramming toward immune activation, pathogen clearance and homeostasis, with early signs of molecular priming observed at the 3 h exposure timepoint.

As for the epithelial cells, at 24 h, XAD increased ROS production without affecting phagocytic activity. In contrast, EXT3 and FERM promoted a faster wound closure. These functional effects were, to a certain extent, supported by the information from transcriptomic analysis. XAD-treated epithelial cells were associated with an enrichment of oxidative stress and metabolic pathways, including glutathione metabolic process, cellular response to xenobiotic stimulus, and cellular iron ion homeostasis, suggesting a stress-adaptive response; while EXT3 exposure was associated with pathways linked to cell migration and tissue remodeling. These results indicate that *S. ramosissima* extracts promote a cell type-specific response, with XAD primarily enhancing immune activation in leukocytes and metabolic adaptation in epithelial cells, whereas EXT3 seems to favor epithelial repair processes.

Overall, the *in vitro* observations on *S. ramosissima* point to their potential as functional additives in aquafeeds, possibly capable of enhancing immune competence and enabling epithelial integrity of gill tissues of Atlantic salmon.

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DIETS SUPPLEMENTED WITH *Salicornia ramosissima* MODULATE EUROPEAN SEABASS GUT MICROBIOTA FOLLOWING A BACTERIAL CHALLENGE

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Introduction

Functional feeds are a promising strategy to improve disease resistance in aquaculture. In this context, plant extracts are rich in bioactives, making them an interesting source of compounds capable of modulating fish health and resilience. Particularly interesting are halophytes such as *Salicornia ramosissima*, which are salt tolerant plants capable of growing in unproductive soils. However, there is limited knowledge about the mechanisms of action of such products, specifically their interactions with the gut microbiota of fish and aquatic animals. Hence, in the present study, we evaluated the effects of diets supplemented with halophytes extracts on survival and gut microbiota of European seabass (*Dicentrarchus labrax*).

Materials and Methods

European seabass of 23.56 ± 0.71 g were fed five experimental diets (CTRL; XAD 0.1%; XAD 0.5%; EXT3 0.1%; EXT3 0.5%) for four weeks. At the end of the feeding trial, fish were challenged with *Photobacterium damsela* subsp. *piscicida* (intraperitoneal injection). Mortality was recorded over eight days, and gut microbiota samples were collected 72 hours post-challenge.

Gut microbiota was analysed using 16S rRNA gene amplicon full-length sequencing. Sequences were processed to generate amplicon sequence variants (ASVs) following quality filtering, denoising, and chimera removal, and were taxonomically assigned using the GTDB reference database. Alpha diversity (Shannon index) and beta diversity (Bray-Curtis dissimilarity) were used to assess community structure across treatments. Differential abundance analysis was performed with metagenomeSeq to identify taxa affected by the diets.

Results and Discussion

Fish fed diets supplemented with XAD 0.1% and EXT3 0.1% had a significantly lower mortality, which indicates that halophyte extracts at lower inclusion levels provide a protective effect against *Photobacterium* infection. Microbiota analysis showed no significant differences in alpha or beta diversity between the treatments. However, despite the lack of clear clustering by diet on Bray-Curtis-based ordination, some differences in dispersion were observed, particularly in the XAD 0.1% and Ext3 0.1% groups.

In contrast, differential abundance analysis revealed some significant differences. Fish fed diets supplemented with halophyte extracts showed a reduction in the relative abundance of *Photobacterium*, with the strongest decrease in the XAD 0.1% group. The genus *Vibrio*, which includes several fish pathogens, was also significantly reduced, but only in fish fed the diets with the lower extracts inclusion (0.1%). In contrast, both XAD diets showed increased abundance of *Acinetobacter*, while both extracts promoted taxa such as *Rhodococcus gordoniae* and *Sphingomonas*, which are often considered beneficial or environmentally relevant.

These results suggest that the protective effects of halophyte extracts might be connected with shifts in specific microbial groups, including reductions in potentially pathogenic genera, rather than large changes in overall microbiota diversity. To clarify the biological relevance of these changes, key ASVs are currently being analysed to understand their closest known relatives using BLAST and phylogenetic analysis.

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Moreover, these extracts are being tested as both prophylactic and therapeutic strategies in other aquaculture species, including Atlantic salmon, rainbow trout, and whiteleg shrimp under chronic stress conditions, to understand their broad potential application in the aquaculture industry.

Acknowledgment

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OMEGA—OYSTER MAPPING, ECOMODELLING, AND GROWTH ASSESSMENT.

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Introduction

Shellfish growing areas have for centuries formed a natural part of the seascape and culture of the Island of Ireland, in part due to the large natural sea loughs which provide an ideal growing environment for bivalves such as mussels, oysters, and scallops.

Bivalve shellfish aquaculture is by nature extensive, and farmers typically have some challenges with respect to stock control, environmental variables, pathogens, and other parts of the business process.

Management of shellfish growing areas is a complex problem: at the core of management is the knowledge of the types, locations, and areas of shellfish culture. The application of models to evaluate carrying capacity and environmental effects relies on sound datasets with respect to stocked areas, stocking density, and other input variables.

Fig 1 shows extensive cultivation of rock oyster (*Magallana gigas*) on trestles in Lough Foyle. Because the culture is organically extractive, rock oysters compete with the native oyster (*Ostrea edulis*) fishery.

Blue mussels (*Mytilus edulis*) were also traditionally grown in the Foyle but at present this is not a key economic resource or a major ecosystem driver.

Over the last fifty years, the industry has changed significantly, in part due to internationalisation, both in terms of Dutch and French entry into the island of Ireland and in terms of the export and branding of Irish product, which is now well recognised in demanding and distant markets such as China.

The expansion of rock oyster cultivation in the Foyle, driven in part by the resistance to oyster Herpes virus at lower water temperatures, has led to stakeholder disputes over the carrying capacity of the lough for different bivalves. The Oyster Management, Ecomodelling, and Growth Assessment (OMEGA) project was developed to: (i) determine the role of Pacific oysters in partitioning the food resource; and (ii) contribute to a fuller understanding of sustainable carrying capacity and top-down eutrophication control in Lough Foyle.



Fig 1. Pacific oyster cultivation in Lough Foyle a transboundary system on the island of Ireland.

(Continued on next page)



Fig 2. Application of machine-learning algorithms showing (left) the accurate distinction between empty trestles and those with oyster bags; and (right) the calculated skill for accurate identification of stocked and unstocked oyster bags.

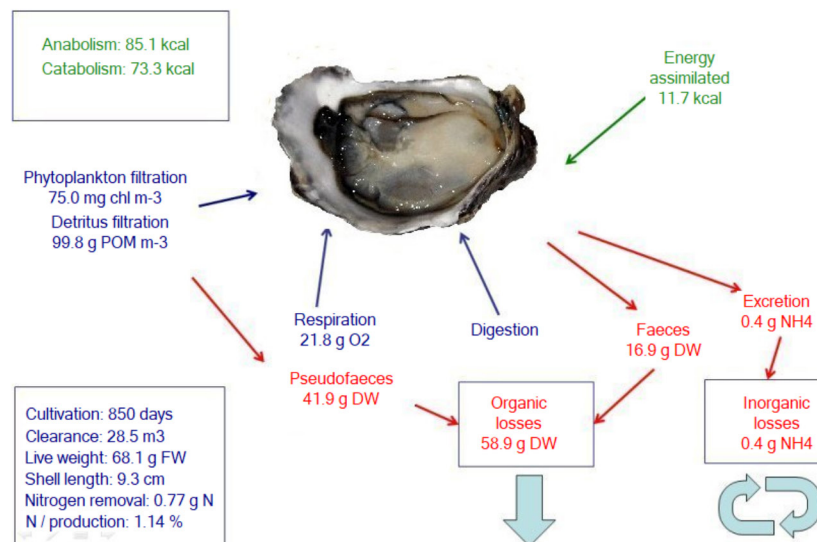


Fig 3. Simulated Pacific oyster growth and environmental effects using the AquaShell model.

Methods

This paper identifies some of the key methodologies used to address these objectives. The first of these, and the main focus of this paper, was the combined use of remote sensing, in particular drone and satellite images, with machine-learning techniques to train algorithms to accurately identify and count oyster bags, which are typically smaller than 1 m². Such algorithms must be able to distinguish between stocked and unstocked trestle areas.

The resulting registry was stored in the AquaScape platform (<https://longline.co.uk/aquascape/>), a web-based geospatial framework that has been used to map about 3.5 million aquaculture structures throughout the world, corresponding to thirty million tonnes of annual production.

In parallel, the well-known EcoWin ecological model, which has been applied to a number of sea loughs on the island of Ireland (e.g. Ferreira et al., 2008; Nunes et al., 2011; Ferreira et al., 2023) was used to simulate bivalve growth and environmental effects, with an emphasis on depletion of algae and organic detritus, to examine various scenarios of future industry development.

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The accurate assessment of the actual area occupied by Pacific oyster and the geographic distribution of the structures used for cultivation was a substantial improvement to the existing carrying capacity modelling of Lough Foyle, providing a robust tool for stakeholder engagement, scenario review, and policy definition.

Results and Discussion

Fig 2 shows an example of the results obtained for image analysis of Pacific oyster culture. Not all the estuarine area can be mapped by drones due to security restrictions—satellite imagery is not as accurate for bag counts. This is a caveat for other shellfish growing areas.

Drone images allowed the mapping of individual bags and the identification of trestles without bags, whereas satellite imagery can only map groups of trestles. In the present work, drone imagery was available for two-thirds of the area (12.31 km²) and resulted in an estimation of a trestle area of 198,693 m², with a corresponding bag count of 272,897.

The total trestle area determined by remote sensing and machine learning was 272,437 m², that contained an estimated 323,935 bags; on average, 53% of the trestle area contained no bags—empty bags were removed by the growers. The ongoing application of the EcoWin model will provide detailed outcomes for carrying capacity, but simple calculations were made using the data acquired to date and the AquaShell bivalve growth model (e.g. Woźniacka et al., 2026).

Fig 3 shows the application of AquaShell to simulate one Pacific oyster, using environmental drivers extracted from the EcoWin model. The outputs were used to scale up the production and environmental effects, considering a stocking density of 80 oysters per bag and a culture period of 850 days. This would result in an annualised production of about 780 tonnes live weight and a corresponding drawdown of 830 kg of chlorophyll.

These numbers do not account for food depletion; EcoWin results will provide a clearer picture of the role of Pacific oysters in partitioning the food resource and of their contribution to top-down control of eutrophication.

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TOXICITY OF A FUNGAL BIOINPUT WITH BIOHERBICIDAL POTENTIAL IN A NON TARGET AQUACULTURE ORGANISM AND ITS RELATIONSHIP WITH FOOD SAFETY

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Introduction

The increasing and often indiscriminate use of synthetic pesticides has caused significant environmental impacts, including soil and water contamination, changes in biological communities, and adverse effects on the health of animals directly or indirectly exposed to these compounds (Pereira et al., 2023). In this context, microbial bioherbicides have emerged as promising alternatives to conventional herbicides because they provide effective mechanisms for weed control while potentially exhibiting lower environmental persistence and impact (Hasan et al., 2021). Among these products, fungal bioinputs consisting of enzymatic extracts capable of degrading plant cell wall components are particularly noteworthy. Although they exhibit herbicidal activity and potential application in more sustainable agricultural systems, their environmental introduction requires careful assessment of ecological safety. The effectiveness of a bioherbicide against target organisms does not, by itself, ensure its environmental safety. Therefore, ecotoxicological assays using non-target organisms that are widely used in aquaculture are essential for identifying possible lethal and sublethal effects (Alves et al., 2024). Amphibians are particularly relevant in ecotoxicological assessments because their life cycles are associated with both aquatic and terrestrial environments, their skin is highly permeable, and they are highly sensitive to changes in environmental quality. Tadpoles of *Lithobates catesbeianus* (bullfrog), besides being commercially produced for human consumption, are widely used as experimental models in toxicity studies because of their hardiness, ease of handling, and ability to exhibit physiological and biochemical changes in response to contaminant exposure (Cunha & Delariva, 2009; Ikari et al., 2024). Therefore, the present study aimed to preliminarily investigate the acute toxicity of a pure fungal microbial bioinput with bioherbicidal potential through the in vivo exposure of *L. catesbeianus* tadpoles, seeking to identify concentrations that do not produce lethal effects in non-target organisms used in aquaculture.

Materials and Methods

Lithobates catesbeianus tadpoles with a mean body weight of 2.52 ± 0.28 g were randomly distributed among experimental aquaria at a density of four animals per liter. The toxicity assay was conducted under semi-static conditions for 192 hours, following the recommendations of ASTM standard E729-23e1 (ASTM, 2023), with complete renewal of the water and exposure solutions after 96 hours. Reverse-osmosis water reconstituted with sodium bicarbonate (NaHCO_3), calcium sulfate dihydrate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), magnesium sulfate (MgSO_4), and potassium chloride (KCl) was used as the exposure medium. During the experimental period, the physical and chemical water parameters were monitored and presented the following mean values: alkalinity of 37.49 ± 2.94 mg/L as CaCO_3 , hardness of 40.98 ± 1.86 mg/L as CaCO_3 , pH of 7.74 ± 0.03 , total ammonia of 0.02 mg/L, electrical conductivity of 339.85 ± 156.55 $\mu\text{S}/\text{cm}$, nitrite of 0.65 ± 0.09 $\mu\text{L}/\text{L}$, dissolved oxygen of 6.7 ± 0.4 mg/L, and temperature of 23.79 ± 1.63 °C. The experimental design consisted of four treatments conducted simultaneously in duplicate: a negative control containing only reconstituted water, Treatment 1 containing 3% pure bioinput, Treatment 2 containing 6% pure bioinput, and Treatment 3 containing 12% pure bioinput. Samples were collected at 0, 96, and 192 hours, with one animal collected from each treatment at each sampling time. Fresh blood was used for the immediate determination of glucose levels using the commercially available Accu-ChekTM Performa system. The liver was subsequently removed, weighed, and processed for the quantification of total protein and total immunoglobulin concentrations using an adapted Lowry/BCA method, as described by Bach et al. (2018).

Results and Discussion

At 96 hours, differences among treatments were observed for all variables analyzed. The 12% concentration resulted in the highest blood glucose level and the lowest hepatic protein concentration and peroxidase activity, whereas the 3% treatment showed the highest protein concentration and peroxidase activity. At 192 hours, blood glucose levels did not differ among groups; however, the 3%, 6%, and 12% treatments maintained lower hepatic protein concentrations than the control. Phenolic compound levels varied among treatments and over time, without showing a linear concentration–response relationship. Peroxidase activity exhibited the most consistent alterations, with reduced values in the exposed groups and the lowest activity in the 12% treatment, even after the increase observed between 96 and 192 hours in some groups. Overall, the results indicate that the bioinput induced sublethal biochemical changes, particularly in protein metabolism and antioxidant response, with more pronounced effects at the highest concentrations. Further studies are required to define environmentally safe concentrations.

(Continued on next page)

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THE INFLUENCE OF LAND-BASED PRODUCTION ENVIRONMENTS ON CARDIAC HEALTH AND FUNCTIONS IN ATLANTIC SALMON

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Introduction

Recirculating aquaculture systems (RAS) have emerged as a promising alternative to the traditional flow-through (FT) system for land-based rearing of Atlantic salmon, offering advantages regarding water usage, biosecurity and control of environmental parameters. However, RAS is often associated with higher concentrations of metabolites and residues in the system, which lowers water quality and potentially increases the vulnerability of fish to multifactorial stressors encountered later in the marine phase. Climate change has become a significant concern in sea-based production, as it can intensify adverse environmental conditions. Elevated water temperatures, reduced oxygen levels, and exposure to jellyfish blooms have been shown to negatively affect fish growth and welfare, yet limited research has focused on the combined effects of multiple stressors. The study examines how production environments, particularly RAS and FT, influence the cardiac health and function of Atlantic salmon, and whether these systems affect susceptibility to climate-related stressors during the marine phase. The findings provide insight for developing climate adaptation strategies and reducing fish morbidity and mortality in aquaculture.

Materials and methods

For the Main Trial, Atlantic salmon parr were divided to be reared in FT or RAS. Fish were subjected to 5 weeks of continuous illumination and a gradual salinity increase to induce smoltification, with heart samples being collected 1 day and 14 days after. Seawater transfer was simulated, and the fish were divided into Control and Test groups. The Test groups were subjected to a stress test that included a gradual increase in water temperature from 12°C to 17°C (1°C per day), followed by a decrease in dissolved oxygen (DO) to 60% for 24 hours, and, lastly, exposure to macerated jellyfish. Hearts were sampled 1 day and 5 days post-exposure. Additionally, 9 fish from each group were selected for a 10-minute electrocardiogram (ECG) assessment, during which hypoxic bradycardia was induced by ceasing artificial gill ventilation in anaesthetised fish. Heart samples were sectioned into atrium, bulbus arteriosus, and ventricle, and differences in the expression of genes related to inflammation, thermal stress, angiogenesis, cardiac growth/remodelling, and hypoxia were analysed by RT-qPCR. Histology and immunohistochemistry were also conducted.

Results

The gene expression profile of the Main Trial showed statistically significant differences between the FT and RAS production environments in atrium and bulbus arteriosus samples, specifically in *ctgf* and *cyr61*, both markers of angiogenesis, with higher relative expression in the RAS groups. In contrast, ventricle samples showed no significant upregulations related to the production systems. Multiple significant differences between the two sampling points were identified across all heart compartments in cardiac growth and remodelling-related genes, including *colla* and *anp*, as well as the previously mentioned angiogenesis markers. In the climate change stress challenge, only one statistically significant upregulation in the RAS group was noted in *heat shock protein 70*, a thermal stress-related gene, within bulbus arteriosus samples. Atrium and ventricle samples showed similar expression patterns in both the FT and RAS environments. Significant differences between sampling points or between the Control and Test groups were frequent across all heart compartments for angiogenesis, thermal stress, and cardiac growth/remodelling markers. The relative expression patterns were maintained throughout the three heart regions, especially in genes associated with inflammation, thermal stress and hypoxia. Results from the ECG assessment revealed no differences between the FT and RAS groups. Induced hypoxic bradycardia was also comparable between FT and RAS, however, in the Test group, the bradycardic response was attenuated one day following jellyfish exposure, with signs of recovery evident 5 days post-exposure. Histology and immunohistochemistry samples are currently being analysed.

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Discussion

Results from the gene expression analysis showed only minor differences between the FT and RAS production environments, with similar trends in relative expression observed across all three heart compartments and in both trials. This consistency was particularly evident for genes related to inflammation, thermal stress, cardiac growth and remodelling, and hypoxia. These preliminary findings suggest that land-based RAS, under the conditions in the experimental set-up, does not compromise cardiac health in Atlantic salmon, nor does it increase susceptibility to climate-related stressors encountered during the marine phase, compared with FT rearing.

Acknowledgment

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ASSESSING A REDUCED-FISHMEAL ORGANIC DIET IN GILTHEAD SEABREAM (*Sparus aurata*): COMPARATIVE RESPONSES ACROSS TWO GENETIC LINES

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Introduction

Organic aquaculture requires feeds that meet strict standards for fish quality, animal welfare, and regulatory compliance. In 2020, it produced 74,000 tonnes (6.4% of EU aquaculture), with gilthead seabream and European sea bass contributing only 1.5% (Estévez & Vasilaki, 2023). Its expansion is limited by strict organic rules, including scarce availability of certified feed ingredients, especially fishmeal, and the ban on synthetic amino acids, which increase costs and reduce formulation flexibility. Alternative proteins such as land-based animal meals and single cell proteins are therefore being explored to overcome these constraints (Sarmiento et al., 2025; Oliva-Teles & Gonçalves, 2001; Marchi et al., 2023). This study evaluated growth performance and quality in two gilthead seabream (*Sparus aurata*) genetic lines (fast-growing and standard) fed a novel organic diet featuring partial fishmeal replacement with hydrolysed yeast extract and inclusion of organic poultry meal.

Materials and Methods

Three isoproteic (43%) and isolipidic (17%) diets (conventional (CC), commercial organic (CO), novel organic (NO)) were tested in a 3 × 2 factorial design across eighteen 800-L tanks in a controlled RAS. The NO diet reduced fishmeal, partially replaced by hydrolysed yeast extract, and included organic poultry meal as an alternative protein source. Fish (133.5 ± 0.25 g) were PIT-tagged, reared under optimal conditions (23–24 °C; salinity 28–33 ppt; dissolved oxygen ~100%; pH 7.8–8.2; 12L:12D), and fed ad libitum until tripling in weight over 94 days. Growth performance, biometric indices, and feed efficiency were evaluated at tank level. Fillet composition and quality, gene expression (muscle, liver, and bone), and plasma biochemistry were analysed in subsampled fish to assess overall responses.

Figure

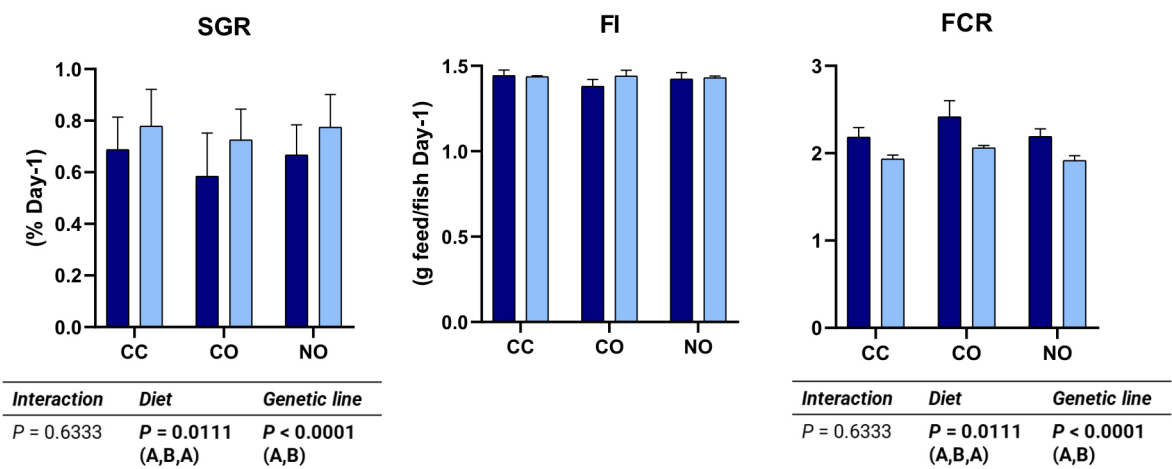


Fig. 1: Growth performance parameters: final body weight (FBW), specific growth rate (SGR), and feed conversion ratio (FCR) as affected by diet (CC, CO, NO) and genetic line (standard, in blue vs fast-growing, in light blue). Two-way ANOVA results with *P*-values reported in the figure.

Results and Discussion

Preliminary results showed that SGR, FCR, and final body weight were significantly affected by both diet and genetic line, with a post-hoc A,B,A pattern (CC, CO, NO). Feed intake was unaffected, whereas the fast-growing line exhibited improved FCR (Fig.1). Somatic indices (HSI and MFI) were unaffected across treatments, while condition factor was higher in the fast-growing line. Fillet quality was mainly diet-driven, with firmness highest in CO and lowest in NO, while no consistent differences were observed for pH or colour. These results align with previous studies showing that well-balanced organic diets can match conventional performance despite reduced fishmeal and absence of syntetic amino acids, while genetic background remains a key determinant of growth efficiency (Estévez & Vasilaki, 2023; Sarmiento et al., 2025) with fillet quality largely unaffected, except for minor diet-related differences in texture (Sarmiento et al., 2025) .

Conclusion

Reducing fishmeal through partial replacement with hydrolysed yeast extract, alongside organic poultry meal as an alternative protein source, sustains growth performance and product quality in gilthead seabream, reinforcing the scalability of EU organic aquaculture.

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THE EXPERIMENTAL AQUACULTURE FACILITY AT NMFRI AS A PLATFORM FOR RESEARCH ON THE ECOLOGY AND RESTORATION OF NORTHERN PIKE *Esox lucius*

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The population of northern pike in the Baltic Sea has declined markedly over the past decade in many regions, with a particularly extreme example observed in Puck Bay, where the species has nearly disappeared. This situation highlights the urgent need to implement measures aimed at population restoration. Key strategies include stocking with hatchery-reared juveniles as well as the restoration and rehabilitation of spawning habitats. Their effective implementation requires the application of modern aquaculture methods. Such conditions have been provided since 2022 at the Experimental Aquaculture Facility of National Marine Fisheries Research Institute (Gdynia, Poland).

The Experimental Aquaculture Facility enables both experimental and breeding work in the fields of fish biology and ecology, including early life stages. It supports research on species inhabiting both freshwater and saline environments. The facility comprises several recirculating aquaculture systems (RAS) configurations, including hatchery, rearing, and experimental units, with a total working volume of 40 m³. Individual systems are equipped with mechanical filtration (microsieves), biological filtration (Trickling biofilters, moving-bed biofilters), oxygen generation and distribution systems, as well as water cooling and heating systems enabling operation within a temperature range of 5 to >25°C. In addition to tap water supply, the systems are supported by installations that allow the delivery of water with controlled and variable parameters. Key water quality parameters in individual circuits are continuously monitored using the OxyGuard Pacific system and made available online via external computers and mobile devices.

The first project conducted at the facility (PIKE) focuses on the rearing of northern pike (including egg incubation and the larval and juvenile stages) under different conditions, including acclimatization to saline waters for stocking coastal areas of the Baltic Sea. In addition to experimental trials, large-scale fry production for stocking natural environment has also been implemented.

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IMPACT OF SALINITY ON OXYGEN CONSUMPTION, SWIM PERFORMANCE, AND BLOOD PHYSIOLOGY OF BROWN TROUT (*Salmo trutta*)

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While the impact of salinity on euryhaline fish species has been well-documented, there remains a significant knowledge gap concerning the effects on hatchery-derived brown trout (*Salmo trutta*), particularly regarding their physiology and swimming performance. To address this gap, 30 freshwater reared *S. trutta* from a landlocked population were divided into 4 treatment groups, acclimatized to brackish (9.5 – 15.0 ppt) or full-strength seawater (31.3 – 34.6 ppt) and individually tested via critical swimming speed (UCrit) trials within an inter-flow closed respirometry chamber in changing salinity regimes. The effect of salinity was measured on oxygen consumption (MO_2), and swim performance - quantified by total trial time (minutes) and maximum swim speed (m/s) – and blood biochemical markers, specifically osmolality, chloride, glucose, lactate, and cortisol. Significant differences in MO_2 responses to treatments post-trial were observed across brackish water and full-strength seawater treatment groups. Full-strength seawater groups did not exhibit reduced swimming performance during UCrit trials compared to brackish water groups. Plasma osmolality and chloride levels showed no significant differences across the experimental timeline and treatment groups, suggesting that the trout adapted well to the altered salinity among the treatments. Glucose, lactate, and cortisol levels suggested that exercise induced stress, or exhaustion did not significantly contribute to the observed results, implying alternative stressors most likely related to handling and/or the inherent stress response to salinity changes. The results suggest a complex interplay of factors influencing the physiological responses of these hatchery-derived *S. trutta*. More comprehensive studies are therefore necessary to better understand the underlying factors driving salinity tolerance for *S. trutta* in both hatchery and wild conditions.

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PRELIMINARY STUDIES ON THE EFFICACY OF PINE LIPID FRACTION AND WOOD POLYPHENOLS AGAINST THE MAIN BACTERIAL PATHOGENS AFFECTING FARMED FISH IN THE MEDITERRANEAN REGION

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Introduction and methodology

Since their discovery, antibiotics have been used to treat bacterial infections in human and veterinary medicine. Unfortunately, their excessive and often inappropriate use has led to the development of antibiotic resistance. This means that certain antibacterial molecules may be less effective, or may no longer be effective, because bacteria have developed the ability to counteract their mechanism of action by acquiring or developing resistance genes.

Applied to the livestock sector, this means that the effectiveness of treatments for bacterial diseases may be reduced, and the associated costs of treatment and mortality may increase leading to significant economic losses. And Mediterranean aquaculture is no exception.

Currently, in addition to vaccination, research is being conducted into probiotic microorganisms and new natural compounds to help facing bacterial infections, whilst reducing the use of antibiotics. The aim of this study was to evaluate the in vitro the growth-inhibiting effects activity of substances as pine lipid fraction and wood polyphenols against some of the most relevant bacterial fish pathogens of Mediterranean aquaculture. This study was conducted in triplicate on 90 strains belonging to eight different bacterial species, such as *Vibrio harveyi*, *Tenacibaculum maritimum*, *Lactococcus garvieae*, *Flavobacterium psychrophilum*, *Photobacterium damsela subsp. piscicida*, *Aeromonas veronii*, *Aeromonas salmonicida*, *Yersinia ruckeri*. Following the Clinical and Laboratory Standards Institute (CLSI) guidelines with some modifications, broth microdilutions in 96-well plates were performed. A 0.5 McFarland suspension of the microorganism under investigation was brought into contact with different concentrations (tested range: 0.0049 – 2.50%) of pine lipid fraction and wood polyphenols to determine the Minimal Inhibitory Concentration (MIC) and the Minimal Bactericidal Concentration (MBC). At the same time for each tested strain, the determination of MIC values against routine applied antimicrobials molecules in aquaculture was also performed.

Results and discussion

Tested plant substances, in particular the pine lipid fraction, demonstrated in vitro activity against bacterial fish pathogens. Promising results in terms of MIC and MBC have been obtained, suggesting the potential beneficial role of investigated compounds if applied in aquaculture sector. In vivo assays will be necessary to confirm these preliminary results and to further support the eventual use of these environmentally friendly substances as an alternative to antibiotics in aquaculture.

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RNA SEQUENCING REVEALS MARKERS OF IMMUNITY IN ATLANTIC SALMON (*Salmo salar*) VACCINATED AGAINST *Tenacibaculum finnmarkense*

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Introduction

Fish viral and bacterial diseases cause substantial losses for the Norwegian aquaculture sector. While vaccination and selective breeding provide protection against many of these threats, there are still a number for which limited preventative measures are available including one causal agent of ulcerative skin disease, *Tenacibaculum finnmarkense*. The aim of study was to evaluate the effectiveness and mode of action of monovalent inactivated vaccines against *T. finnmarkense* in Atlantic salmon (*Salmo salar*).

Methods

Three different vaccine regimes comprising both water-based immersion and oil- based IP vaccines were tested and immune system metrics assessed at various points in the vaccination process. Initial results demonstrated a specific IgM response against *T. finnmarkense* in all IP vaccinated fish and significant differences in mortality were observed between unvaccinated control fish and vaccinated fish, allowing us to select 100 representative head kidney and thymus samples from different timepoints of our best vaccination group.

Results

The transcriptomic data has allowed us to characterise the immune and transcriptional response of Atlantic salmon against this *T. finnmarkense* vaccine, as well as the acute stress response of a placebo vaccination, and to identify biomarkers associated with this response. Additionally, we have identified several differentially expressed genes which can be used in the future as potential disease biomarkers.

MITIGATION OF HARMFUL ALGAL BLOOMS SPECIES THAT AFFECT SCOTTISH AND NORWEGIAN ATLANTIC SALMON FISH FARMS USING MODIFIED CLAYS

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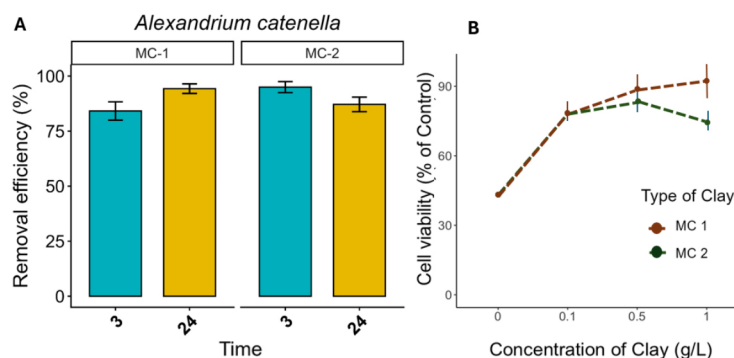
Introduction

Understanding how to control microalgal species that negatively impact Atlantic salmon fish farms in fjord systems is a priority for the industry. Harmful Algal Blooms (HABs) in the waters of Scotland and Norway threaten both local food security and global aquaculture production, and they cause significant fish mortality and economic losses (Davidson et al., 2020). Several algal species can damage gill tissues or release toxic metabolites, compromising fish health and welfare in aquaculture systems (Hallegraeff et al., 2023; Planellas et al., 2026). The frequency, duration, and geographic extent of HAB events across Northern Europe are expected to increase with the changing climate (Murray et al., 2025). This increases the urgency for efficient and cost-effective mitigation strategies. At the moment, there are no widely adopted techniques in the salmon industry that are effective at mitigating the adverse effects of HAB (Anderson et al., 2025).

A promising technology is the application of modified clays (MC), which can adhere to algal cells, resulting in the flocculation and sinking of harmful species (Yu et al., 2017). These clays are modified with poly-aluminium chloride, which confers a positive surface charge on the materials, thereby altering their structure and increasing the attraction between the materials and the cells (Yu et al., 2017; Song et al., 2021). Furthermore, it is suggested that these MCs have the capacity to degrade algal toxins. In addition, MCs have been used in large-scale HABs across China, Malaysia and Turkey including open waters for fish aquaculture and enclosed culture ponds.

This study is the first in Europe to investigate the efficiency of commercially available MC (from the IOCAS) in removing seven selected HAB species from the water column under laboratory conditions at fjord-relevant temperatures and salinities. Furthermore, it will evaluate the potential impact of MCs on fish gill cell lines (RTgill-W1) as a proxy for fish toxicity. In addition, using this bioassays to assess unknown toxins and HPLC-MS/MS to detect known toxins. The study will also examine whether these materials can mitigate the toxic effects of harmful algae by adsorbing toxins.

Figure 1: Effect of MCs on *Alexandrium catenella*.



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Methodology

We performed a flocc-and-sink experiment, in which cultures of algae were mixed with two different clays (MC-1 and MC-2) at a final concentration of 0.2 g L^{-1} , and cell removal was measured after 3 and 24 hours. These experiments targeted HAB species that cause physical damage to farmed fish: *Chaetoceros curvisetus* (CCAP 1010/12), and toxic species for fish: *Alexandrium catenella* (CCAP 1119/17), *Alexandrium minutum* (CCAP 1119/49), *Karenia mikimotoi* (RCC10703), *Karlodinium veneficum* (NORCCA K-1634), *Chrysochromulina leadbeateri* (NORCCA UIO394), and *Prymnesium parvum* (CCAP 946/7). At the same time, use the RTgill-W1 cell line as a bioassay to determine whether these clays are cytotoxic (and potentially toxic to fish) and whether they can adsorb the toxic compounds produced by the algae. This experiment was conducted on the cell line exposed to MC-1 and MC-2 alone at different concentrations and then assessed whether the MCs could mitigate the effects of the three species, *A. catenella*, *K. mikimotoi* and *C. leadbeateri*.

Results and Discussion

The results showed that, on a laboratory scale, both MCs reduced cell density in the water column by at least 50% for all taxa after 24 hours. For thecate dinoflagellates and diatoms, the removal efficiency was more than 75% in both types of clay at 3 hours (Figure 1A). In addition, athecate dinoflagellates show increased removal efficiency after 24 hours. Removal efficiency varied significantly among phytoplankton species and over time (Yu et al., 2017). Additionally, the RTgill-W1 bioassay MC-1 didn't show any reduction in cell viability in any of the concentrations tested. Furthermore, the cell line bioassay results suggest that MCs could adsorb toxic compounds produced by *Alexandrium catenella*, increasing the cell viability at higher concentrations of MCs (Figure 1B). This indicates sorption capacity of MCs for harmful algal compounds, as previously suggested by Song et al. (2021).

A) Removal efficiency over time of two type of MCs.

B) Cell viability of RT-gill W1 on exposure of *Alexandrium catenella* supernatant with MCs

Overall, modified clays demonstrate considerable potential as a mitigation strategy against several HABs that affect fish. Concomitantly, it can adsorb toxic compounds, thereby increasing gill cell viability. Nevertheless, further research is required to evaluate their environmental safety and potential impacts on non-target organisms in European aquaculture, such as farmed fish.

Acknowledgments

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ADVANCING INFECTION MODELS FOR EMERGING BACTERIAL PATHOGENS IN EUROPEAN SEA BASS (*Dicentrarchus labrax*) UNDER CONTROLLED AND REAL PRODUCTION CONDITIONS

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Introduction

Bacterial diseases represent a major constraint for Mediterranean aquaculture, particularly in European sea bass (*Dicentrarchus labrax*), where increased farming intensity and environmental changes are contributing to the emergence of pathogenic species (Reverte et al., 2020). Among these, *Aeromonas veronii* has been widely recognized as an emerging pathogen associated with disease outbreaks in European sea bass, particularly under elevated temperature conditions (Tanrikul & Dinçtürk, 2021). Recent efforts have therefore focused on the development of reproducible infection models for this species, enabling the evaluation of preventive strategies under controlled conditions. In this context, the incorporation of multiple isolates from different outbreaks has become essential, as intraspecific variability in virulence has been reported for *Aeromonas spp.*, potentially affecting disease outcomes and model reproducibility (Smyrli et al., 2019). Accordingly, this study expands previous work by including new *A. veronii* strains isolated from European sea bass in different geographical and epidemiological contexts, allowing a broader assessment of pathogenicity. In parallel, *Lactococcus garvieae* has emerged as a relevant pathogen in marine aquaculture, with recent outbreaks reported in European sea bass farms in Mediterranean countries. This pathogen is associated with septicemia and high mortality rates, particularly under stressful environmental conditions such as elevated temperature and fluctuating salinity (Fouz et al., 2025). Despite its growing importance, infection models under realistic production conditions remain limited.

Therefore, the present study aims to (i) refine and expand infection models for *A. veronii* using multiple isolates and (ii) develop and validate infection models for *L. garvieae* under both controlled (Recirculating Aquaculture Systems - RAS) and semi-natural (estuarine pond) conditions representative of Mediterranean aquaculture systems where the effects of climate change are more evident.

Materials and methods

Bacterial isolates of *A. veronii* and *L. garvieae* were obtained from diseased European sea bass during field outbreaks and identified using MALDI-TOF and qPCR. Strains were cryopreserved and incorporated into the CTAQUA collection. Experimental challenges were conducted using European sea bass at pre-ongrowing and ongrowing stages. For *A. veronii*, multiple isolates were tested using intraperitoneal (IP) injection and bath immersion to evaluate dose-response relationships and inter-strain variability. For *L. garvieae*, infections by intraperitoneal (IP) injection were conducted under different experimental conditions. On the one hand, trials were performed under standard farming conditions representative of current production situation for this species and pathogens. On the other hand, additional trials were carried out under more extreme environmental conditions, including elevated temperature and salinity fluctuations, designed to simulate those typically observed in estuarine pond systems (esteros) in southern Spain, which are increasingly influenced by climate change. This approach allowed the evaluation of pathogen behavior under both current farming conditions and climate change-driven stress scenarios.

For bath infections, the water level in the system tanks was reduced or 10 L buckets were used, depending on fish size. Under these conditions, fish were exposed to the pathogen for 1 hour at the different tested concentrations, while aeration and animal welfare were continuously monitored. For IP injection challenges, 0.1 mL of bacterial suspension per fish was administered at the desired concentration. Prior to injection, fish were deeply anesthetized using benzocaine. All trials included control groups (not exposed to the pathogen during bath infections or inoculated with Phosphate Buffered Saline (PBS) during IP injection infections) and were performed in triplicate. Mortality, culture parameters, clinical signs, and bacterial re-isolation were recorded.

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Results and discussion

For *A. veronii*, dose-response relationships were consistent across the different isolates, although mortality rates differed significantly among strains (Smyrli et al., 2019). The results confirm that *A. veronii* isolates exhibit variable virulence depending on virulence factors of the strain, the route of administration, but also fish origin, size and batch, highlighting the importance of incorporating multiple strains and conditions for the development of robust infection models that allow the evaluation of different preventive measures or more versatile and universal treatments, capable of covering the pathogenicity of different strains. *L. garvieae* induced a septicemic disease in European sea bass, with infection dynamics strongly influenced by environmental conditions (Salogni et al., 2024). Higher temperatures and salinity fluctuations, simulating estuarine system conditions, affected both mortality and disease progression. Comparative analyses between standard and extreme conditions revealed more heterogeneous and severe outcomes under semi-natural conditions. Overall, these findings demonstrate the need to integrate environmental variability into infection models to better reflect real farming conditions and improve the evaluation of preventive strategies.

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SEA OASIS PIRAN: PILOTING REGENERATIVE AQUACULTURE AND HABITAT ENHANCEMENT IN THE NORTHERN ADRIATIC

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Introduction

Coastal marine aquaculture is increasingly expected to provide food while reducing pressures on marine ecosystems and adapting to environmental change. At the same time, many production systems remain dominated by monoculture and offer limited opportunities for species diversification. This creates a need for approaches that move beyond impact mitigation towards multifunctional aquaculture capable of combining production with ecological functions. Sea Oasis Piran is a field-scale pilot established by the YouSea Institute in the Gulf of Piran, northern Adriatic Sea, within an existing licensed marine aquaculture area. The initiative investigates whether purpose-designed underwater structures can provide a physical and ecological basis for the development of Integrated Multi-Trophic Aquaculture (IMTA), while simultaneously increasing habitat complexity and supporting marine biodiversity (Giangrande et al., 2021).

The long-term objective is to develop and scientifically evaluate a model in which aquaculture infrastructure contributes to both production and ecological functions. In particular, Sea Oasis Piran explores the potential for integrating low-trophic organisms and habitat-forming structures into a regenerative IMTA approach applicable to small-scale Mediterranean aquaculture sites.

Pilot design and monitoring framework

Sea Oasis Piran currently comprises four complementary underwater structures deployed between 2024 and 2026, representing contrasting structural complexity, substrate types and potential ecological and aquaculture functions. The structures incorporate different surfaces using concrete and limestone, open metal and mesh configurations, a three-dimensional biomimetic structure designed to increase structural complexity, and modular rope elements intended to support settlement and potential cultivation of low-trophic marine organisms, including commercial bivalve species.

Rather than functioning as artificial underwater structures spatially and operationally separated from aquaculture, the structures are conceived as experimental components of an IMTA system. Their different geometries and substrates provide an opportunity to investigate how design influences biological colonisation, habitat use, natural recruitment and compatibility with aquaculture operations. The structures therefore function both as habitat-enhancement units and as experimental platforms for evaluating design features relevant to future regenerative aquaculture applications.

Systematic quantitative monitoring is currently being established. The monitoring framework will combine repeated underwater photographic, video and remotely operated vehicle surveys with documentation of sessile colonisation and mobile fauna. Major taxonomic and functional groups will be recorded and changes in community composition and habitat use followed over time. Where low-trophic cultivation trials are implemented, biological performance will be assessed alongside relevant environmental conditions. Structural stability, maintenance requirements and compatibility with routine aquaculture activities will also form part of the evaluation.

Preliminary results

Although systematic quantitative data collection is only beginning, repeated field observations following deployment show rapid and taxonomically diverse biological colonisation of the structures. Observed sessile and associated organisms include sponges, tunicates, bivalves such as oysters, mussels and small scallops, polychaetes, bryozoans and several groups of algae. Egg masses of different gastropods and squid have also been recorded on the structures, indicating their use as reproductive substrate. Multiple wild fish and crab species have been observed around and within the structures, which aligns with previous studies (Ardizzone et al., 1989); these observations are treated as evidence of habitat use rather than as an aquaculture production outcome.

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The spontaneous settlement of locally occurring commercial bivalves (e.g. *Mimachlamys varia*, *Ostrea* sp., *Aequipecten opercularis*) and other low-trophic organisms (small sea spider *Maja crispata*) is particularly relevant to the aquaculture component of the pilot. These observations provide a basis for testing whether purpose-designed structures can facilitate natural recruitment and potentially support the collection of locally sourced seed for future low-trophic cultivation. The present observations are qualitative and are being used to refine sampling design and identify priority variables for subsequent quantitative assessment.

Significance

By integrating aquaculture potential, habitat complexity and ecological monitoring within the same operational marine farming site, Sea Oasis Piran explores a transition from predominantly monoculture-based aquaculture towards more diversified, regenerative and ecosystem-based production systems. The pilot will assess whether purpose-designed structures can simultaneously support aquaculture functions, habitat use and the natural recruitment of locally occurring low-trophic species. This could contribute to aquaculture diversification by enabling evaluation of locally recruited native species whose recruitment occurs under local environmental conditions, while reducing dependence on single-species production.

The pilot is expected to identify synergies and trade-offs between production-related activities and biodiversity enhancement and to provide a field-scale framework for evaluating multifunctional aquaculture structures. Sea Oasis Piran therefore offers a platform for developing and testing an ecosystem-based aquaculture model with potential relevance to other small-scale aquaculture sites in the Adriatic and wider Mediterranean region.

Acknowledgment

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FONDA FISH GARDEN: AQUACULTURE TOURISM AS A MODEL FOR EDUCATION, PUBLIC ENGAGEMENT AND FARM DIVERSIFICATION

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Introduction

European aquaculture is increasingly challenged to remain economically viable while strengthening its environmental performance, public acceptance and connection with coastal communities. For small and medium-sized farms, diversification does not necessarily require increasing production volume; it can also involve creating complementary services that add value to existing production, knowledge and place. Aquaculture tourism offers such an opportunity by opening working farms to visitors and connecting seafood production with education, local gastronomy and direct producer-consumer interaction. Recent studies indicate that exposure to aquaculture during marine tourism can support learning and more positive attitudes towards aquaculture-tourism integration (Kim et al., 2020; Budhathoki et al., 2025).

Fonda Fish Garden in the Gulf of Piran, northern Adriatic Sea, provides a long-running Slovenian example of this approach. Established as a marine fish farm in 2003, the site gradually developed a tourism and educational programme around its core aquaculture activity. This contribution presents Fonda Fish Garden as a practice-based case study of how aquaculture tourism can diversify a farm's activities while creating a direct setting for communicating aquaculture practices, seafood provenance and the relationship between farming, the marine environment and the local coastal economy.

Aquaculture tourism model

The tourism experience is built around the operation of a real, working marine farm rather than a separate visitor attraction. Guided experiences combine a boat journey from Seča to the offshore farming area with interpretation of the Gulf of Piran, the Sečovlje Salina landscape, the fishing reserve and local marine ecosystems. At the farm, visitors are introduced to the biological and technical principles of sea bass farming, juvenile selection, feeding, growth, stocking density, fish welfare, environmental care, traceability and product quality. Manual fish feeding is demonstrated and the return route includes observation of mussel cultivation. The visit is complemented by tasting locally produced seafood together with regional products, thereby linking primary production with gastronomy and place-based identity. Additional formats developed around the same knowledge base include seafood preparation and cooking workshops and smaller educational experiences.

This format transforms farm infrastructure and day-to-day husbandry into an interpretive environment. The producer is not only a supplier of fish but also a source of first-hand knowledge, while visitors can observe where and how farmed seafood is produced and ask questions directly. The approach therefore creates opportunities to address common misconceptions about aquaculture, explain production trade-offs and distinguish evidence-based sustainability practices from generic sustainability claims. Importantly, the tourism activity remains dependent on the operational and environmental limits of the farm: group size, navigation safety, weather conditions and farming activities determine when and how visits can take place.

Building on this sustainable tourism model, the programme is progressively incorporating elements of regenerative tourism through cooperation with local marine-conservation non-governmental organisations, particularly the YouSea Institute. This collaboration connects aquaculture interpretation with ocean literacy, biodiversity conservation, responsible behaviour at sea and opportunities for public participation in marine stewardship. The aim is not only to minimise tourism impacts, but also to use the farm visit as an entry point for strengthening visitors' understanding of and engagement with the protection of the local marine environment.

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Evidence as a recognised good practice

The model has received repeated external recognition for innovation and educational value. The tourism concept received the Slovenian Snovalec recognition in 2014 and was a Sejalec finalist in 2015. In 2019, 'An Unforgettable Day at the Fonda Fish Farm' received the Jakob International Award in the category of new sustainable forms of fishing and wildlife-related tourism; the jury specifically highlighted the combination of fish farming with tourism, education and awareness-raising. The experience was subsequently included among Slovenia Unique Experiences, the national collection of boutique five-star experiences curated by the Slovenian Tourist Board.

Independent destination research also provides quantitative support for its perceived experiential value. In a 2024 study of unique tourism products in Piran and Portorož, based on 230 valid questionnaires, Fonda Fish Garden received a mean uniqueness score of 4.00 out of 5 (SD = 0.844). Its perceived uniqueness was positively associated with consumer expectations ($B = 0.152$, $p = 0.028$), while increasing knowledge of the local offer, traditions and history was the highest-rated expectation across the tourism products examined (Čivre et al., 2024). These data do not measure learning outcomes from the farm visit itself, but they support the relevance of authenticity, local character and educational content in the visitor proposition.

Significance for aquaculture diversification

The Fonda Fish Garden case shows how aquaculture tourism can create value from assets that already exist within a farm: production knowledge, skilled personnel, farm infrastructure, local ecological context, product identity and seafood quality. For smaller coastal farms, this can provide a complementary revenue stream and strengthen links with tourism, gastronomy and local producers without making higher biomass production the only route to economic development. At the same time, direct public access can make aquaculture more visible and understandable and may contribute to social licence by increasing transparency and opportunities for dialogue.

Transferability depends on more than opening a farm to visitors. The experience must remain authentic, safe and compatible with production; interpretation should be delivered by people who understand both the biology and the farming process; and the tourism product should connect aquaculture with the ecological and cultural characteristics of its location. Fonda Fish Garden demonstrates that, when these elements are integrated, a working aquaculture site can function simultaneously as a food-production area, a learning environment and a tourism product. The model therefore offers a practical example of activity diversification for small-scale European aquaculture, with the potential to evolve from sustainable towards more regenerative tourism through collaboration with marine-conservation actors. It also provides a platform for future research on visitor learning, perception, social acceptance and the economic contribution of aquaculture tourism.

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THE INVASIVE BLUE CRAB *Callinectes sapidus* AS AN OPPORTUNITY FOR THE BLUE ECONOMY SECTORS IN SLOVENIA

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Introduction

The Atlantic blue crab (*Callinectes sapidus*) has emerged as a major invasive threat to Mediterranean coastal ecosystems, fisheries and aquaculture. In the northern Adriatic, its rapid expansion has been associated with severe predation on farmed bivalves, particularly Manila clam (*Ruditapes philippinarum*). In two major shellfish-farming lagoons of the Po Delta, losses of farmed clams reached up to 100%, affecting both seed and market-sized individuals and demonstrating the species' potential to seriously disrupt established aquaculture production (Chiesa et al., 2025). In the Bay of Piran, Slovenia (northern Adriatic Sea), *C. sapidus* is now routinely caught for experimental removal in the Jernejev Canal in Seča, an ecologically important coastal area within a Natura 2000 site and the management area of the Sečovlje Salina Nature Park. Because fishing within the protected area requires a specific permit, the resulting catches cannot currently be placed on the market, creating a regulatory barrier to economically supported removal. Since 2025, within the framework of the Delicious Invasion project, the authors have tested an integrated management approach combining systematic removal fishing, culinary valorisation and ocean-literacy activities. The approach explores whether an invasive predator threatening coastal ecosystems, fisheries and aquaculture can simultaneously be transformed into a locally valorised seafood resource, creating an economic incentive for sustained population control. Consumer research elsewhere in the Mediterranean indicates substantial willingness to support blue-crab removal through its purchase and consumption (Marchessaux et al., 2024), suggesting that market valorisation may complement ecological management.

Materials and Methods

As *C. sapidus* occurs within canals that fall under a protected area, fishing was conducted under written permission for scientific research purposes from the protected area management body and Ministry of Agriculture, Forestry and Food. Between October 2025 and July 2026, blue crabs were removed from the Jernejev Canal during 41 fishing/monitoring events, using non-invasive, highly selective gear (a baited line and a hand-held net or baited crab trap) to eliminate bycatch and impact on the surrounding habitat. All individuals ($n = 1176$) were sexed and staged (juvenile, adult, ovigerous), measured (carapace width, carapace length) and weighed, and screened for epibionts. In parallel, the removal programme was linked to two complementary valorisation and engagement pathways: (i) restaurant-based culinary events testing the potential of blue crab as a locally marketable seafood resource while building consumer awareness and acceptance; and (ii) school-based fishing and cooking workshops combining direct participation in invasive-species removal with ocean-literacy education on marine invasions, biodiversity and aquaculture impacts.

Results

The sampled population was predominantly adult (979 of 1174 individuals, 83%), with 181 juveniles and 14 ovigerous females, and was male-biased overall (875 males vs 299 females). Carapace width ranged 52–191 mm (mean 131.8 ± 24.2 mm) and weight 7–473 g (mean 169.5 ± 79.7 g). Epibionts, predominantly filamentous algae, were recorded on 34.9% of individuals (410/1176). Size and population composition varied over the ten-month study period. Mean carapace width increased from 122 mm in October 2025 to a peak of 159 mm in June 2026, before declining to 146 mm in July 2026. Females outnumbered males in the catch only in February 2026 (67%). Catch per fishing event also declined over the study period, from a maximum of 61 individuals in October 2025 to single-digit catches by mid-2026 (range 6–61 overall); while consistent with the cumulative effect of sustained removal fishing, a standardised effort measure would be needed to confirm this as a true abundance trend. Restaurant tasting events and school fishing and cooking workshops were delivered regularly throughout the same period. To date, 6 local restaurants have participated, developing 15 blue-crab recipes and organising dedicated promotional events, such as blue-crab days and the inclusion of blue crab in regular seasonal menus, to raise consumer awareness and demand. In parallel, 15 educational activities engaged 225 participants, mostly primary and secondary school children, their parents and teachers. Participants reported high satisfaction and increased awareness of the blue crab invasion. The initiative also received substantial media coverage, further increasing public visibility of the issue.

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Discussion

This study demonstrates that structured, permit-based removal can generate a substantial blue-crab catch while simultaneously supporting culinary valorisation and public engagement. More importantly, sustained removal may represent a precautionary measure against the kind of pressure this predator has already caused elsewhere in the northern Adriatic (Chiesa et al., 2025). The Slovenian case therefore represents an opportunity to develop management measures before impacts on production systems reach the scale observed in neighbouring Italy. However, the socio-economic potential of such an approach is presently constrained by a legislative gap: under current Slovenian fisheries and nature-conservation legislation, catches taken under a scientific/management permit within a protected area cannot be commercially sold, while commercial fishing is generally restricted in these areas. This separates the ecological incentive to maximise removal from the economic incentive that could help sustain fishing effort. Market valorisation should not be regarded as a substitute for invasive-species management, particularly because experience from heavily affected production areas shows that economic returns from blue-crab exploitation may not compensate for losses to shellfish aquaculture. Rather, it could provide an additional mechanism for supporting targeted and continuous removal. However, any such incentive carries the inherent risk of sustaining demand for, rather than reducing, the invasive population, so derogations should permit commercial use only for catch verifiably tied to authorised removal effort, not an independent fishery. We therefore recommend: (i) a dedicated legal derogation allowing traceable commercial use of invasive alien species removed from protected areas under authorised management programmes; (ii) coordinated approval between fisheries and nature-conservation authorities; and (iii) development of a national or Adriatic-regional blue-crab management and valorisation strategy linking invasive-species control, protection of shellfish aquaculture, traceability and local seafood value chains.

Acknowledgment

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DIETARY KRILL MEAL MODULATES STRESS AND REDOX RESPONSES TO CHRONIC HYPOXIA IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Reduced dissolved oxygen availability is an increasing challenge for salmonid aquaculture, particularly under climate change scenarios where rising water temperatures decrease oxygen solubility while increasing metabolic demand (Johnston et al., 2025). Chronic hypoxia can impair growth and feed utilization and induce physiological disturbances associated with oxidative stress and activation of stress-response pathways (Lushchak, 2011; Magnoni et al., 2018). Nutritional strategies supporting antioxidant defenses and maintaining redox homeostasis are therefore receiving growing attention (Ciji and Akhtar, 2021). Antarctic krill meal (KM) is a functional ingredient rich in antioxidant compounds, including astaxanthin, selenium and vitamins, and has been associated with beneficial effects on fish performance and health (Kaur et al., 2022). However, its role in modulating physiological stress and redox responses under hypoxic conditions remains poorly documented. Therefore, this study investigated the effects of dietary KM supplementation on stress-related and antioxidant responses of juvenile rainbow trout exposed to chronic hypoxia.

Materials and methods

Juvenile rainbow trout (37 ± 1 g) were fed a control diet (CTRL) or diets supplemented with 5% (KM5), 7.5% (KM7) or 10% (KM10) krill meal for 12 weeks under normoxic conditions. Fish were subsequently exposed to chronic hypoxia (5.9–6.5 mg/L dissolved oxygen) for 3 weeks while continuing to receive their respective diets. Plasma stress indicators (cortisol and glucose), total antioxidant capacity (TAC), hepatic glutathione redox status and expression of antioxidant- and hypoxia-related genes were assessed. Effects of diet and rearing condition were analysed by ANOVA.

Results and discussion

Chronic hypoxia significantly increased plasma cortisol concentrations (+33%, Fig. 1) and moderately elevated plasma glucose levels (+4.3%), while inducing a shift towards a more oxidized hepatic redox status, as reflected by increased GSSG levels and a reduced GSH/GSSG ratio. Hypoxia also upregulated the expression of several antioxidant-related genes, including *sod1*, *cat*, *gr* and *gclc*, indicating activation of compensatory antioxidant mechanisms. Dietary krill meal supplementation attenuated the cortisol response under hypoxia, while plasma total antioxidant capacity remained consistently 6–10% higher in KM-fed fish than in CTRL fish, irrespective of oxygenation conditions (Fig. 1). Similar responses were observed for both Q1 and Q2 antioxidant fractions. Dietary treatment also modulated hepatic redox regulation, with significant effects on GSSG content, GSH/GSSG ratio and expression of genes involved in glutathione metabolism. In particular, expression of *gr* was enhanced in fish fed the highest krill meal level, supporting a role of KM in maintaining redox homeostasis. Interestingly, expression of the hypoxia-responsive marker *egln3a* remained unchanged after 3 weeks of hypoxia, possibly reflecting physiological acclimation to the moderate hypoxic challenge and a transient activation of canonical hypoxia-signalling pathways. Together, these findings indicate that krill meal supplementation enhances systemic antioxidant capacity while modulating hepatic redox regulation, thereby contributing to improved physiological adaptation to chronic hypoxic stress.

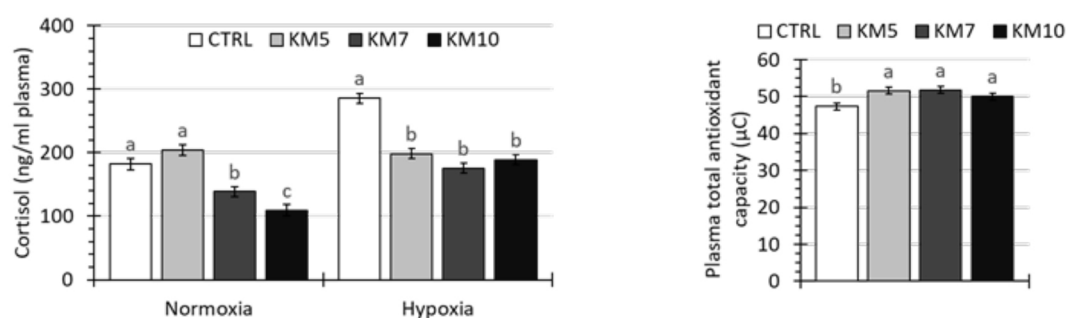


Figure 1. Plasma cortisol and total antioxidant capacity of rainbow trout fed the experimental diets under normoxic and hypoxic conditions.

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Conclusion

These findings demonstrate that dietary krill meal supplementation modulates both physiological stress and redox responses in rainbow trout exposed to chronic hypoxia. By attenuating cortisol response, enhancing systemic antioxidant capacity and influencing hepatic redox regulation, krill meal appears to support physiological robustness under environmental stress conditions. These results highlight the potential of krill meal as a functional ingredient to improve resilience of farmed salmonids facing increasingly variable oxygen conditions.

Acknowledgment

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EFFECT OF AN ANTIBIOTIC THERAPY AND A BICULTURE WITH THE RUSSIAN STURGEON ON PIKEPERCH GROWTH AND BACTERIAL COMMUNITIES IN RAS

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Introduction

In recent decades, the monoculture farming of pikeperch (*Sander lucioperca*) has expanded, primarily in Europe and Central Asia, relying on recirculating aquaculture systems (RAS), land-based aquaculture systems (Pěnka et al., 2025). However, while RAS farming offers many advantages (water savings, control of rearing conditions, etc.), this system also has drawbacks, notably high production costs that increase when energy prices rise. Furthermore, pikeperch remains a species highly sensitive to stress related to rearing conditions and various handling procedures (Baekelandt et al., 2018). This vulnerability can lead to greater susceptibility to pathogens, particularly opportunistic bacteria, and consequently necessitate the use of antibiotic treatment (survey towards the European Percid Fish Culture Group, 2024). Moreover, due to its specific behavior and biological preferences, pikeperch reared in monoculture does not make optimal use of available resources in tank (diet, water volume and depth) and polyculture, with another species, would have a benefit effect on rearing conditions and fish welfare. Therefore, the aim of our study is to evaluate the effects of antibiotic therapy and polyculture with the Russian sturgeon *Acipenser gueldenstaedtii*, a highly complementary species (Pěnka et al., 2024), on zootechnical performances of both species reared in RAS and the consequences on bacterial communities of the system, which is influenced by fish species and play major roles in RAS (Changey et al., 2025).

Materials and methods

Our study was carried out at the Experimental Aquaculture Platform (PEA) of the University of Lorraine using 12 temperature-lighting-controlled independent RAS. The initial acclimatization of 110 kg of pikeperch (individual weight of 200-300 g, from La Ferme Intégrale, La Baume-d'Hostun, France) and 50 kg of Russian sturgeon (individual weight of 75-250 g from Belgium Quality Fish, Dottignies, Belgium) was done in separate specific tanks during september 2024. After this period, the batch of pikeperch was divided in two separate RAS which only one received an antibiotic therapy for 10 days (15 g of Trisulmix per 100 kg of fish added to the diet). Then fish were transferred to experimental ecotrons on October 4th (10 kg/tank). Four experimental treatments were applied in triplicate: monoculture of pikeperch without previous antibiotic therapy (P), monoculture of pikeperch with previous antibiotic therapy (P+Ab), biculture of pikeperch without previous antibiotic therapy and russain sturgeon (PE) and biculture of pikeperch with previous antibiotic therapy and russain sturgeon (PE+Ab) with 50% of each species for biculture. The experiment lasted two months at a constant temperature (23-24°C) and fishes were fed at 1.2% feeding rate (Percid Grower Le Gouessant, 52% lipids, 15% proteins and 21.4 MJ/kg energy). Water quality was controlled weekly and individual fish growth monthly (30 fish/species/ecotron), in order to characterize the growth performance of each species, biomass gains, and feed efficiency. Bacterial communities of gut, surfaces biofilm, and water was analyzed using 16S rRNA gene metabarcoding analysis.

Results and discussion

At the end of the experimental period, we observed that the mortality was very low and similar in all treatments. However, significant effects were observed on final biomass and biomass gain which were significantly higher in bicultures compared to monocultures. Lower food conversion rates were calculated in bicultures (IC = 1) compared to the monocultures among which the feed conversion ratio was significantly higher in the treatment group “pikeperch monoculture without antibiotic therapy” (IC = 2.5). Considering performances of each species, any significant difference was recorded in their final mean body weight, those of Russian sturgeon were exactly similar (1.15-1.20 kg) whereas for pikeperch, the final mean weight is close to be significantly higher in the mixed culture with antibiotic therapy” treatment compared to the pikeperch monoculture (P-value = 0.0629). We observed that pikeperch feeding behaviour was different in biculture compared to monoculture. Significant effects of antibiotic therapy and fish species on RAS bacterial communities were recorded. Antibiotic therapy induced a sharp reduction in alpha-diversity within the gut of all treated pikeperch, as well as in the water of the corresponding experimental groups. PERMANOVA results for planktonic and sessiles communities demonstrate that

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this effect significantly extends to beta-diversity in the water and in the biofilms ($R^2 = 0.3293$; P-value= $1e-4$; $R^2 = 0.2778$; P-value= $1e-4$ respectively). Notably, the polyculture with or without a previous antibiotic therapy for pikeperch, exhibited the highest similarity in terms of beta diversity for both water and biofilms, (despite remaining significantly different ($R^2 = 0.21758$ p=0.01 for water ; and $R^2 = 0.1116$; p=0.047 for biofilms) suggesting a greater resilience of biofilms formed in polyculture compared to those in monoculture.

Acknowledgments

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PROTEIN SUPPLEMENTATION AS A FUNCTIONAL INGREDIENT TO MODULATE THE ANTERIOR INTESTINAL RESPONSE TO OXIDATIVE STRESS: AN *EX VIVO* APPROACH

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Introduction

In recent years, insect-derived ingredients have emerged as promising sources of bioactive compounds for the development of novel functional ingredients in animal health research. Among edible insects, *Tenebrio molitor* is one of the most extensively studied species, with its larval stage being the first insect approved for human consumption in the European Union. In contrast, the adult stage is generally regarded as a low-value by-product due to its higher chitin content, despite retaining a high protein content (approximately 50%) together with other potentially valuable bioactive compounds. The valorisation of adult *T. molitor* biomass therefore represents an opportunity to improve resource efficiency within insect production systems, reduce waste, and support the transition towards a circular bioeconomy. This study aimed to evaluate the effects of proteins obtained from adult *T. molitor* on immune and oxidative status using an ex vivo intestinal explant model of European seabass (*Dicentrarchus labrax*).

Material and Methods

An initial viability screening was conducted to evaluate the effect of five protein inclusion levels (0.5, 2.5, 7.5, 15, and 20%) on anterior intestinal explants from European seabass (*Dicentrarchus labrax*). Tissue viability was assessed via the MTT assay (Erickson-DiRenzo et al., 2015), with 50% DMSO used as a non-viable control, by measuring formazan absorbance at 570 nm. Based on the screening results, the 0.5%, 1% and 2.5% levels were selected for subsequent evaluation. Explants were pre-incubated for 1 h in pre-treatment medium, Dulbecco's Modified Eagle Medium (DMEM) supplemented with 500 U penicillin-streptomycin, 10% fetal bovine serum (FBS), 2 mM glutamine, and 5.5 mM glucose. The medium was then replaced with fresh DMEM containing 100 U penicillin-streptomycin, 10% FBS, 2 mM glutamine, and 5.5 mM glucose, supplemented with the selected protein concentrations or no protein (control), and incubated for 2 h. Following this incubation, pre-challenge samples were collected and stored in RNeasy lysis buffer at -80°C , while the remaining explants were subjected to a challenge with hydrogen peroxide (H_2O_2) for 2 h under the same medium conditions. After this period, explants were collected and stored in RNeasy lysis buffer at -80°C for gene expression analysis.

Results

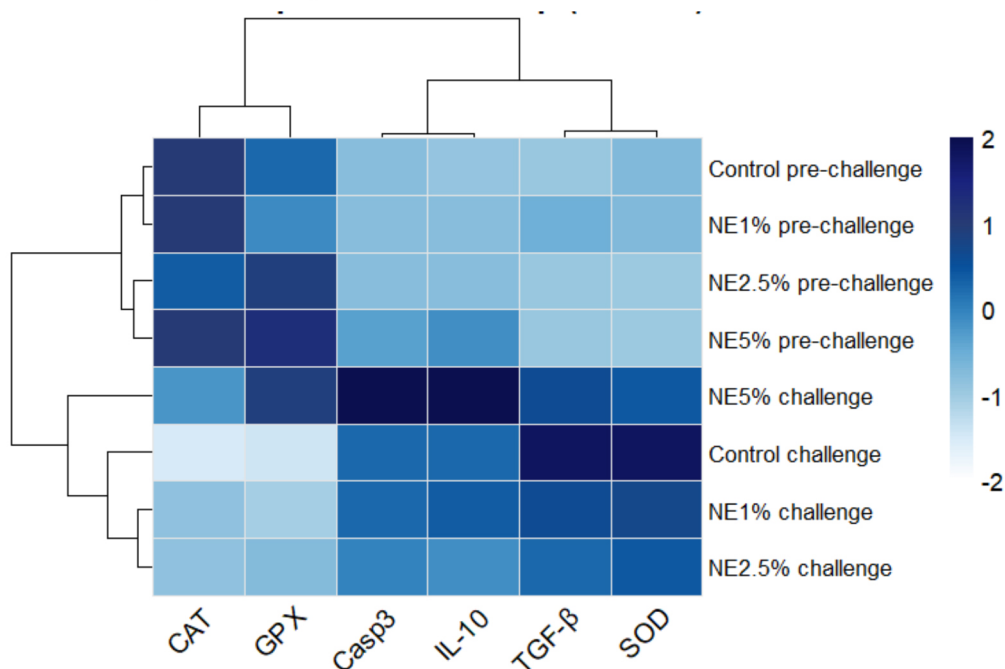
The viability assay showed that all the tested protein supplementations were safe for the tissue. So, three concentrations were chosen to perform the ex vivo assay.

Gene expression was assessed in the anterior intestine before and after a hydrogen peroxide challenge. Compared with pre-challenge levels, H_2O_2 exposure upregulated *casp-3*, *tgf- β* , *il-10*, and *sod*, while downregulating *cat* and *gpx*. Polynomial contrast analysis showed that increasing protein supplementation of the incubation medium induced quadratic responses in *casp-3* and *il-10* expression and linear responses in *sod* and *gpx* expression, whereas *tgf- β* expression showed no significant linear or quadratic trends. Furthermore, explants incubated in medium supplemented with 5% protein exhibited higher expression of *casp-3*, *il-10*, and *gpx* than the other treatments (Figure 1).

These findings demonstrate that proteins derived from adult *T. molitor* can modulate intestinal oxidative stress and immune responses in a dose-dependent manner, highlighting their potential as functional ingredients for aquafeeds while contributing to the valorisation of insect production by-products.

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Figure 1. Clustered heatmap of relative expression of immune and oxidative genes in intestinal explants of European seabass.



Acknowledgments

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THE NORWEGIAN SEAWEED CENTRE

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About the seaweed centre

The Norwegian Seaweed Centre is a national research and innovation infrastructure designed to advance sustainable seaweed aquaculture as a strategic contribution to future food, feed, material, and energy systems. In response to global challenges related to population growth, climate change, and increasing pressure on natural resources, the centre aims to develop scalable, environmentally responsible marine biomass production systems that support the transition to a sustainable blue bioeconomy. The centre combines fundamental and applied research with technology development and industry collaboration across the full seaweed value chain, creating a comprehensive platform for innovation and knowledge generation.

Overview of the different nodes.

The Seaweed Centre has a total of six nodes that are thematically divided into onshore facilities, offshore facilities, simulation and monitoring, vessels and logistics, preprocessing and storage as well as processing and products (Figure 1).

The **onshore facility** provide controlled environments for experimental cultivation, enabling systematic investigation of growth conditions and biomass quality. These facilities support early-stage development and testing before scaling to open-sea environments. The **sea facilities** constitute a core component of the centre, offering access to offshore and nearshore cultivation sites where large-scale production systems can be evaluated under realistic environmental conditions. These sites allow for the assessment of structural designs, deployment strategies, and ecosystem interactions associated with seaweed farming.

To support data-driven decision-making and system optimization, the centre includes a dedicated node for **simulation and monitoring**. This node integrate environmental monitoring, sensor technologies, and numerical models to analyse oceanographic conditions, biomass performance, and environmental impacts. Such tools are essential for improving site selection, predicting production outcomes, and ensuring sustainable operation. **Vessels and logistics** node provide the necessary maritime infrastructure to enable deployment, maintenance, harvesting, and transport operations, bridging the gap between experimental research and industrial-scale application.

Beyond cultivation, the Norwegian Seaweed Centre addresses post-harvest challenges through nodes focused on **preprocessing and storage**, as well as **processing and product development**. These nodes support research on handling, preservation, and stabilization of seaweed biomass, as well as its conversion into value-added products for food, feed, biobased materials, and other industrial applications. This end-to-end perspective enables holistic evaluation of technical feasibility, resource efficiency, and sustainability across the entire value chain.

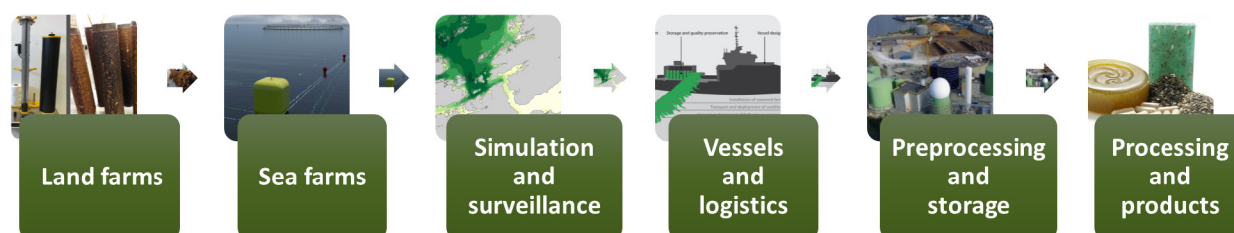


Figure 1: An overview of the infrastructure and the six different nodes

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Through coordinated projects spanning integrated multi-trophic aquaculture (IMTA), blue carbon potential, environmental sustainability, and commercialization pathways, the Norwegian Seaweed Centre serves as a national and international hub for collaboration. By bringing together infrastructure, interdisciplinary competence, and stakeholder engagement, the centre aims to accelerate innovation in seaweed aquaculture and contribute to climate mitigation, coastal development, and long-term ecosystem resilience within a sustainable marine bioeconomy

Acknowledgment

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<https://norwegianseaweedcentre.com/>



POLYFREE – EFFECTIVE METHODS FOR THE CONTROL OF INVASIVE PARASITIC POLYDORA WORMS IN OYSTER AQUACULTURE

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Introduction

Shell-boring polychaetes of the genus *Polydora* and related taxa (Polydorini) are among the most important pests of farmed molluscs (Simon and Sato-Okoshi, 2015). These worms bore into shells, inducing the formation of mud blisters filled with sediment and faecal material (Blake and Evans, 1973; Read 2010). In oysters, infestation can induce health problems, higher susceptibility to environmental stressors, mortality, and influence negatively product quality and its market value (Korringa, 1954). Numerous non-native polydorin pests have been transported worldwide through the translocation of infected oyster adults and juveniles (Fossi et al., 2026). Recently, new non-native pests *P. caeca* and *P. websteri* have been reported in oyster farms in the Adriatic Sea, Italy (Mikac et al., 2025). Despite their recognized impact, the management of *Polydora* infestations remains challenging. A wide range of physical and chemical treatments has been proposed in different experimental and geographical contexts, often showing variable effectiveness and limitations related to host safety or operational feasibility. This study aims to identify and experimentally test the most effective and applicable protocols for the removal of shell-boring polychaetes in Italian oyster farms within the framework of the PolyFREE project.

Materials and Methods

A literature-based assessment of removal treatments for shell-boring polychaetes was conducted through a systematic search of peer-reviewed articles, technical reports and grey literature. Protocols were comparatively evaluated using a multi-criteria scoring system based on: (i) quality of the test, (ii) efficacy against the parasite, (iii) host safety, (iv) operational applicability, and (v) environmental and economic sustainability. Experimental trials will be conducted across four seasons on naturally infested oysters from two Italian farms, under controlled laboratory conditions, using replicated treatment groups and untreated controls. Treatment efficacy will be assessed by evaluating the abundance of residual live *Polydora* remaining inside oysters after their treatment and a few-days stabilization period. Worms will be extracted by immersion in a phenol solution. Oyster mortality and treatment-associated stress will also be monitored during the post-treatment stabilization period. Subsequently, the most promising protocol(s) will be tested under field conditions in oyster farming facilities located in the Emilia–Romagna region.

Results and perspectives

The literature review identified a broad range of physical and chemical treatments for the control of shell-boring polychaetes in aquaculture systems worldwide. A total of 37 publications were examined and comparatively evaluated. The evaluation highlighted substantial variability among treatments, particularly regarding treatment efficacy, oyster tolerance, and practical applicability under farming conditions. Based on the overall assessment, three non-chemical physical treatments were selected for experimental validation: (1) freshwater immersion followed by air drying, (2) hypersaline immersion followed by air drying, and (3) prolonged desiccation. Results of the experimental phase, currently underway, will be presented at the conference. They will provide the first comparative validation of *Polydora* treatment protocols under controlled laboratory and field conditions in oyster farming systems of the Emilia–Romagna region. Information on successful treatment protocols will be communicated to oyster producers, and workshops will be organized if needed. The PolyFREE project is expected to contribute to the development of standardized, sustainable and low-impact management strategies against shell-boring polychaete infestations in Mediterranean oyster aquaculture.

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Acknowledgment

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PREDOMINANCE OF A *Lactococcus garvieae* SUBTYPE PATHOGENIC TO EUROPEAN SEA BASS (*Dicentrarchus labrax*) AND GILTHEAD SEBREAM (*Sparus aurata*) IN MEDITERRANEAN AQUACULTURE

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Introduction

Lactococcus garvieae (Lg) has recently emerged as a major pathogen in the farming of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*), the main species in the Mediterranean aquaculture (Salogni et al., 2024; Fouz et al., 2026). *Lactococcus garvieae* is a common fish pathogen that can cause significant economic losses in several fresh and saltwater species. Despite the extensive range of hosts, *L. garvieae* infection in sea bass (*Dicentrarchus labrax*). In parallel, infections caused by *Lg* have also been reported in marine fish farms in Japan, and the isolated strains have been classified into different genetic groups: types I, II and III (the newly emerging *Lg* serotype III) (Araki et al., 2024). However, no information is available as to whether the European strains belong to the same groups as those described in Japan. The aim of this study was to characterize the *Lg* strains isolated in Spanish and Italian marine farms according to the Japanese classification system and to investigate serological variability within this pathogenic marine group.

Materials and methods

Bacterial strains: a collection of strains isolated from diseased sea bass and sea bream in Spanish and Italian farms from 2023 to 2025 was identified using PCR targeting the Glycosyltransferase family 4 coding protein gene of *L. garvieae* (Saticioglu et al., 2023). Reference strains CECT 4828 and CECT 5807, isolated from yellowtail (*Seriola quinqueradiata*) and rainbow trout (*Oncorhynchus mykiss*), respectively, were also included. All strains were routinely grown on Trypticasein Soy Agar (TSA) or TSA supplemented with 4 % of defibrinated sheep blood (TSAB) at 28 °C for 24-48 h.

Genetic classification: Bacterial genomic DNA was extracted using the boiling technique (Fouz et al., 2026) and stored at -20 °C. To determine the genetic type to which the marine isolates belong, the multiplex PCR developed by Araki et al. (2024) was carried out. Strains of type I, II (recognised as *L. formosensis*) or III generate fragments of 262 bp, 1004 bp or 500 bp, respectively.

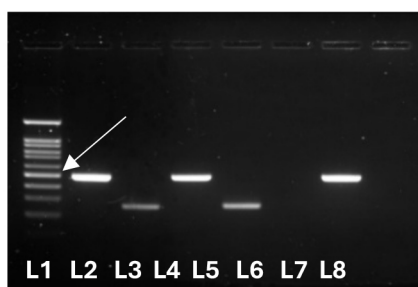


Figure 1. Agarose gel electrophoresis of the PCR products obtained from DNA samples of pure cultures of *Lactococcus garvieae* (Lg). Amplicons of the *Lg* specific types I or II (262 bp or 500 bp, respectively) can be visualised. From left to right: **Lane (L) 1**, 100bp molecular weight DNA ladder (M); **L2**: *Lg* G1 (Spanish sea bass); **L3**: *Lg* G2 (Spanish sea bass); **L4**: *Lg* IZ811 (Italian sea bream); **L5**: *Lg* CECT 4828; **L6**: *Lg* CECT 5807; **L7**: *Lg* IZ574 (Italian sea bass) **L8**: negative control (no DNA sample).

Table 1. Genetic and serological classification of *Lactococcus garvieae* (Lg) strains isolated from diseased sea bass or sea bream in Spanish and Italian farms.

Lg strains (n)	Geographic origin	Strains of PCR-genetic type*		Strains of serotype**	
		I	III	G1	G2
12	Spain	2	10	10	2
39	Italy	0	39	35	4

*Genetic type according to Araki et al. (2024). ** Serotypes described here.

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Serology: rabbit polyclonal antisera were raised against formalin-killed whole cells of strains representative of the two putative serotypes (Ser), corresponding to Spanish isolates G1 and G2 (Fouz et al., 2026). Then, slide agglutination with whole cell suspensions (10^8 colony forming units (CFU)/mL) was performed (Pavlinec et al., 2022) a disease that threatens the long-term sustainability of aquaculture. Current research shows that the Mediterranean strains of *V. harveyi* are serologically heterogeneous, though research comparing the traits of different strains is scarce. This study aims to describe the biochemical, physiological and genetic characteristics of three serologically different strains of *V. harveyi* isolated from farmed European Sea bass (*Dicentrarchus labrax*).

Results and discussion

It was confirmed that the strains isolated from diseased sea bass and seabream corresponded to *L. garvieae* using species-specific PCR. Furthermore, most produced a 500 bp fragment following multiplex PCR, as would be expected from type III strains. Only two Spanish sea bass isolates (G2.1 and G2.2) (Fouz et al., 2026) resulted positive with the type I primers and generated a 262 bp fragment (Fig. 1 and Table 1). CECT 4828 strain tested positive for type I, whilst CECT 5807 tested negative for all three types, as expected (Aoki et al., 2024) (Fig. 1). Thus, most European marine *Lg* strains were classified as type III.

Agglutination tests revealed that the *Lg* isolates from marine species classified as genetic type III belonged to serotype G1 (Table 1). The two Spanish isolates classified as type I reacted positively with the serum raised against the G2 strain and only few Italian type III strains gave positive with this serum (Table 1).

Conclusions

Most strains of *L. garvieae* isolated from diseased sea bass and seabream in Spanish and Italian farms are classified as type III by PCR multiplex and belong to a dominant serotype within the emerging marine group, serotype G1. This study highlights the serological diversity of *Lg* strains responsible for lactococcosis in marine fish, a growing threat to aquaculture that underscores the urgent need to update surveillance and control strategies.

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Acknowledgements

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EXTERNAL EXPOSURE AND RETURN CONNECTEDNESS IN THE BRAZILIAN TILAPIA VALUE CHAIN

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Introduction

Brazilian tilapia is the most important aquaculture species produced in the country and plays a strategic role in domestic fish supply. Although exports account for only a small share of total production, the sector has experienced increasing integration with international markets, particularly through exports of value-added products such as fresh fillets. At the same time, the production chain is organized around distinct regional hubs that differ in production systems, governance structures, and market relationships. These characteristics create a complex environment in which domestic and external factors may jointly influence price dynamics across the value chain.

Monthly data from January 2022 to December 2025 were collected for farmgate prices in the main production hubs (Grandes Lagos, Norte do Paraná, and Oeste do Paraná), wholesale prices, export prices for fresh fillets and frozen whole tilapia, and the BRL/USD exchange rate. All series were transformed into logarithmic returns and tested for stationarity prior to analysis. Return connectedness was assessed using the Diebold and Yilmaz framework, based on the Generalized Forecast Error Variance Decomposition (GFEVD) derived from a Vector Autoregressive (VAR) model (Diebold & Yilmaz, 2012). Total, directional, and net connectedness measures were estimated to quantify the intensity and direction of shock transmission among markets and to identify the main transmitters and receivers of return spillovers within the Brazilian tilapia value chain.

Results and Discussion

The results revealed a strongly interconnected spillover structure across the Brazilian tilapia value chain, with the Total Connectedness Index remaining above 70% during most of the study period. As illustrated in Figure 1, fresh fillet export prices emerged as the dominant net transmitter of shocks, followed by the BRL/USD exchange rate and the Oeste do Paraná production hub. In contrast, wholesale prices, Grandes Lagos, and frozen whole tilapia exports acted primarily as net receivers. The dynamic analysis further showed that connectedness intensified during the sample period, indicating that a large share of price variability was driven by cross-market interactions rather than by idiosyncratic shocks. Robustness analyses confirmed the stability of these findings across alternative forecast horizons.

The network representation (Figure 1) highlights the central role of fresh fillets markets and exchange-rate dynamics in transmitting shocks throughout the system. These findings suggest that, despite its predominantly domestic orientation, the Brazilian tilapia sector remains exposed to external market conditions through export-price and currency channels. Fresh fillets and frozen whole tilapia displayed distinct spillover patterns, indicating functional segmentation between export products and highlighting the greater influence of value-added products on price transmission. Regional differences were also evident, with Oeste do Paraná occupying a central role in domestic shock propagation, potentially reflecting its stronger cooperative structure and higher degree of production coordination. Conversely, Grandes Lagos exhibited greater susceptibility to incoming shocks. Overall, the results emphasize the importance of governance arrangements, market organization, export activities, and exchange-rate dynamics in shaping risk transmission within aquaculture value chains.

Acknowledgment

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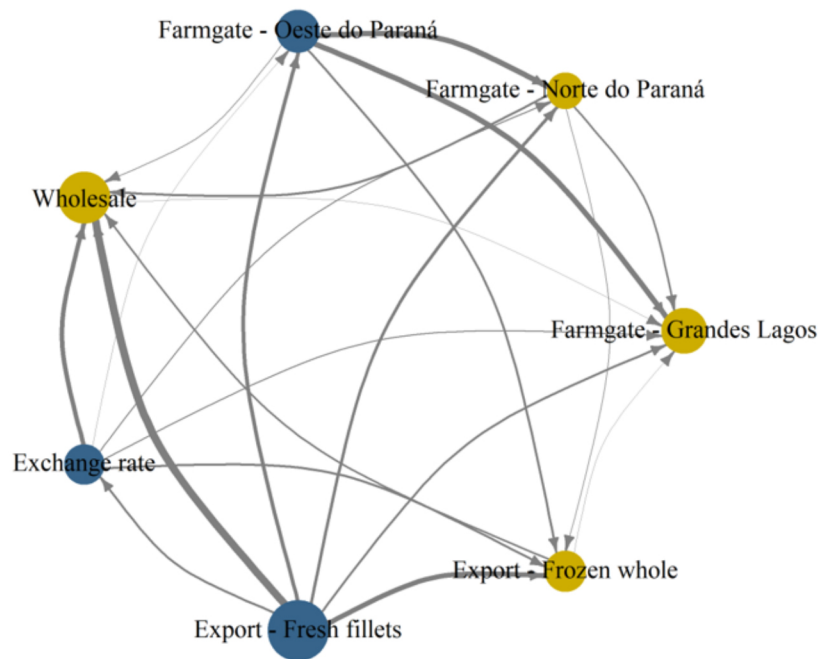


Figure 1 - Network representation of return spillovers in the Brazilian tilapia market system. Nodes represent markets and direct edges indicate the transmission of shocks from source to receiver; node sizes represent the magnitude of shock transmitted or received; node colours reflect if they are receivers (yellow) or transmitter (blue); and edge thickness is proportional to the magnitude of spillovers.

SnappArt 360 E-SENSE: AI MODEL FOR AUTOMATED EGG STAGE DETECTION TO SUPPORT MODERN HATCHERY MANAGEMENT

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Introduction

Accurate assessment of spawned eggs number and embryonic development in fish eggs and larvae is a critical step in hatchery management, directly influencing survival rates, production efficiency, and decisionmaking throughout the early life cycle. Current assessment practices in hatcheries are predominantly based on rapid visual inspection and general stage estimation, often complemented by additional manual checks. While these methods are practical, they remain subjective, speciesdependent, and limited in their ability to consistently evaluate developmental quality. This variability reduces comparability across hatcheries and increases reliance on expert interpretation.

To address these limitations, SnappArt 360 E-Sense has been introduced, a species-specific, automated imaging and analysis solution designed to accurately determine the number of the eggs spawned, the embryonic development from fertilization to the early larval stages, but also define the stage of eggs maturation in case of ovarian biopsy by cannulation. Integrated into the SnappArt 360 platform, the E-Sense tool combines high-resolution imaging with advanced image analysis algorithms to automatically identify developmental stages, quantify key morphological features, and generate standardized quality metrics. A species-specific approach ensures that algorithms and reference models are biologically relevant and tailored to distinct embryological patterns.

By replacing subjective evaluation with a consistent, data-driven methodology, SnappArt 360 E-Sense aims to improve accuracy, reduce labor dependency, and enable harmonized embryonic assessment across hatchery operations.

The aim of this study is to explain how an AI model, trained on images labeled by experts, along with digital support systems that can recognize and track egg stages of different species as *Sparus Aurata*, *Dicentrarchus labrax*, *Lates calcarifer*, *Solea Senegalensis*, *Scophthalmus maximus* ex *Psetta maxima*, *Gadus morhua*

In addition to the device, an integrated software platform processes the collected data and generates clear reports on periodic spawning events and corresponding hatching rates. This functionality supports hatchery managers in effectively monitoring broodstock egg production and maintaining operational control.

Materials and Methods

A known volume egg sample collected from the eggs collector was processed and prepared to be easily divided into a 3x4 cell culture plate, with 1ml per cell. Images of the wells are acquired using the SnappArt 360 device, a digital imaging system, consisting of a camera connected to a local edge-computer device based on the Nvidia Jetson Platform developed by Aris in collaboration with INVE. The captured images were transferred to CVAT (Computer vision annotation tool) for annotation by trained experts. The dataset consists of the following classes: viable eggs fertilized, viable eggs embryo (about 20h from fertilization), non viable eggs (degenerated eggs), healthy larvae, dead larvae, membranes, stage A-B-C-D depending on the maturation of the unfertilized eggs in the case of a preliminary maturation check of the oocyte in seabass. These expert-annotated images were used to train and validate an AI model based on the YOLO (You Only Look Once) object detection architecture. Afterwards, this trained model was installed on the SnappArt to be tested in the field by technicians. After deployment on the SnappArt, it requires only basic input from the user, such as tank origin, tank volume, and sample volume. After a fast image capture of each separate well, the SnappArt delivers a comprehensive report detailing the number of viable eggs, the degenerated ones, and the number of eggs per stage (egg fertilized or embryo). An associated data management platform enables long-term tracking and analysis of broodstock production and quality of the spawn, keeping traceability of the hatching rate from fertilization to hatch.

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Results

The accuracy of the Snappart AI system was evaluated by comparing automated counts with manual counts performed by an experienced technician using a stereomicroscope. Manual counting requires high operator expertise to correctly identify viable and degenerated eggs, avoid miscounting, and involves substantial time investment. The time required for manual counting is roughly double that of Snappart360.

In contrast, the Snappart AI system, based on the E-Sense model, allows result verification through realtime image recording and demonstrated 100% correct eggs detection for up to 200 items per well. Overall, the system achieved a total counting accuracy of 99%.

Discussion

The most advanced model is currently applied to fertilized seabream and seabass eggs and provides a broad overview of periodic spawning, supporting effective monitoring and planning of the hatchery.

The detection of the oocyte developmental stage before hormonal induction is another key capability of the system. Within a few minutes, the system provides the percentage distribution of the different egg developmental stages prior to spawning. This enables fast and informed decision-making regarding the most appropriate induction protocol to be applied to the selected breeders.

The automated approach eliminates the need for expert assessment of developmental stages, increasing process standardization and significantly reducing analysis time and accuracy with multiple replicates.

SUBSTRATE AND TIMING ARE KEY TO SUCCESSFUL *Fucus* SEEDING IN A NATURAL BED

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Along the Danish coastline, *Fucus serratus* is a key habitat-forming brown algae. Due to its commercial use (e.g., as food, in pharmaceuticals, and cosmetics), it is widely harvested, yet, unlike Laminariales, rarely cultivated. As a result, knowledge on reproductive phenology, seeding methods, optimal deployment timing, and yields is limited. This study addressed these gaps by evaluating the gamete production and in vitro fertilization success, followed by the monthly deployment of seeded and unseeded substrates (concrete H-blocks and ropes) in Begtrup Vig (Baltic Sea, Denmark) over 1.5 years within a natural *Fucus* spp. bed. Mature *F. serratus* receptacles were present year-round with a peak abundance per individual from August to February. Fertilization success was maximal from October to January with a male:female gamete ratio of >50:1. The seeding on ropes showed low success (maximum 54 individuals m⁻¹ after one year). In comparison, concrete substrates had a density of up to 69,185 ± 9,862 individuals m⁻², particularly when deployed in April. Overall, a deployment in April and May resulted in three months earlier visibility in comparison to other deployments and higher growth rates (up to 9.9% d⁻¹). While *F. serratus* was artificially seeded, *Fucus vesiculosus* relied on natural recruitment. However, *F. vesiculosus* clearly dominated, reaching a maximum yield of 54.26 kg m⁻², outperforming *F. serratus* (maximum 16.19 kg m⁻²) despite not being actively seeded. The study indicates that year-round seeding is possible, but spring seeding is most favourable. Deploying unseeded stones to be seeded by natural recruitment, and following harvested, may be a promising approach to mitigate the impacts of wild harvesting, while rope-based cultivation requires further research.

UPCYCLING MYCOTOXIN CONTAMINATED CARBOHYDRATES IN EU FOOD SYSTEM VIA INSECT BIOREFINERIES

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Introduction

Insect biorefineries are increasingly recognized as a circular solution for sustainable animal feed production, offering an efficient conversion of underutilized organic resources into protein- and lipid-rich biomass. Their environmental and economic benefits, however, depend strongly on the use of low-value or contaminated waste streams rather than conventional feed materials that compete with food production, although the use of such substrates is currently restricted under EU legislation. Among such underexploited resources are carbohydrate-rich food products discarded due to mycotoxin contamination. Here, we investigate the potential of insects to upcycle ochratoxin-contaminated bakery waste into valuable biomass while generating insect-derived products free of mycotoxins.

Materials and Methods

Four-week-old larvae of *Tenebrio molitor* (yellow mealworm) were reared for 12 weeks on five different dietary substrates: (i) 100% wheat bran, used as the commercial control diet; (ii) 100% breadcrumbs derived from mycotoxin-contaminated bakery products (naturally mold-contaminated bread and bread rolls); (iii) 100% commercially produced breadcrumbs (Penam Plc, Czech Republic); (iv) a mixed diet consisting of 50% wheat bran and 50% mycotoxin-contaminated bakery products; and (v) a mixed diet consisting of 50% commercially produced breadcrumbs and 50% mycotoxin-contaminated bakery products. Final larval body weight, survival rate, and Fulton's condition factor were determined. In addition, the initial and final mycotoxin contents of the 100% wheat bran and 100% mycotoxin-contaminated bakery product treatments were analyzed in an accredited laboratory (AGRO-LA LLC, Czech Republic) using HPLC-MS/MS and a semi-accredited protocol (SOP 8.136.) with detection limits as low as $0.05 \mu\text{g kg}^{-1}$.

Results

After 12 weeks of rearing, the ochratoxin-contaminated moldy bread treatment proved to be as suitable a substrate as the commercial control diet, with no significant differences observed in final larval biomass, body weight, survival rate, or Fulton's condition factor ($p > 0.05$). Among all tested substrates, only treatments containing commercially produced breadcrumbs resulted in significantly lower larval body weight and final biomass ($p < 0.05$). The initial ochratoxin concentration in the contaminated substrate was $13.8 \mu\text{g kg}^{-1}$, corresponding to a total ochratoxin load of $0.828 \mu\text{g}$. During the experiment, this load decreased by 51.5% to $0.401 \mu\text{g}$ as a result of substrate conversion by the larvae. Nevertheless, ochratoxin was not detected in larvae reared on the contaminated substrate.

Discussion

Large amounts of fungi- and mycotoxin-contaminated carbohydrate-rich materials (e.g., grains, bread, and tubers) are discarded worldwide each year and are typically composted, biodegraded, incinerated, or landfilled. Insects, however, have evolved to utilize such microbially colonized substrates, and our results support this adaptation by demonstrating the production of mycotoxin-free larval biomass from mycotoxin-contaminated feedstocks, with growth performance comparable to that achieved on a standard uncontaminated rearing substrate. Furthermore, mealworm feeding reduced the overall ochratoxin load by decreasing the amount of contaminated substrate. These findings highlight the potential of future insect biorefineries to upcycle ochratoxin-contaminated carbohydrate streams into valuable insect-derived protein and lipids for animal feed, thereby contributing to a more circular food system.

Despite this potential, current EU legislation classifies insects together with vertebrate livestock as 'farmed animal' or 'food-producing animal', creating barriers to the full utilization of insect bioconversion. Establishing a separate regulatory category for insects, with more flexible regulations for their production as feed for 'farmed animal', would be an important step toward more effective and sustainable insect farming in Europe.

Acknowledgment

The work was funded by DIVERSIFY4Future - Diversifying aquaculture for the future: inclusion of low-trophic species for sustainable, innovative, healthy and circular food systems project has received funding from the European Union's Horizon Europe Research and Innovation Actions, under grant agreement N° 101286791.

EVALUATION OF ANTIMICROBIAL USAGE IN RAINBOW TROUT (*Oncorhynchus mykiss*, Walbaum, 1792) FARMS OF TRENTO AND UDINE PROVINCES (ITALY)

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Introduction

Aquaculture represents a particularly critical sector amongst animal production concerning antimicrobial usage (AMU) and its potential impacts on animal health and the surrounding environment. In Italy, official AMU data are not systematically collected: while some regions have performed the collection in regional datasets, others lack reliable information (Scoppetta et al., 2018). Within Italian aquaculture, rainbow trout holds a central role, as it constitutes the most extensively produced species, placing Italy among the leading European producers. Approximately 70% of national trout production is concentrated in North-eastern Italy where, there is a representative spectrum of structural, managerial and environmental conditions across different farming systems. This research project aims to quantify AMU and to estimate the trend of antibiotic consumption in the provinces of Trento and Udine (primary areas of rainbow trout farming in the North-East of Italy). This activity constitutes a preliminary step towards the development of a standardised national methodology for data collection and a subsequent investigation into the factors influencing the AMU.

Materials and Methods

To define the study population, data from the national livestock registry were used. These data were processed to create lists divided by the provinces involved (Udine and Trento), including all establishments intended for human consumption that kept at least the species rainbow trout. These lists were submitted both to the competent territorial veterinary authorities (ASUIT and ASUFC) and to the trade association (API) for validation. ASUIT and ASUFC provided data on pharmacological treatments carried out in the selected establishments over the three-year period 2022–2024, through access to the national information system which collects all prescriptions issued for farmed animals. Production data, expressed in kilograms of slaughtered and/or marketed fish for each selected establishment, were provided by API, which collects them annually.

AMU was calculated using a dose-based methodology, which makes it possible to take into account the potency of each commercial formulation in relation to a specific treatable biomass, known as the Population Correction Unit (PCU). The data were processed in Define Daily Dose Animal for Italy (DDDAit) to obtain a tailored profile for the commercial products used in Italy. Briefly, the DDDAit of an antimicrobial medicinal product refers to the quantity of active substance, expressed in milligrams, that should be given per kilogram of live body weight each day of treatment, in accordance with the summary of product characteristics (SPC). This metric is the one established during the development of the ClassyFarm management system (it is a national IT platform that collects and processes a significant amount of data relating to animal welfare, biosecurity on farms, antimicrobial consumption and susceptibility, etc.), which currently does not consider fishes but only some terrestrial species. In Italy, the trout slaughter weight varies depending on the type of final product required; for the purposes of this study, we have estimated an average weight of 1 kg. According to the literature (Narbonne et al., 2021), a reasonable treatment weight can be taken as half the slaughter weight meaning in our case 0.5 kg. The total number of fish can be approximated by dividing the annual slaughter biomass by this 1 kg reference weight. This estimated fish count, together with the assumed average treatment weight of 0.5 kg per animal, can then be used to calculate the PCU for each selected establishment.

Results and Discussion

Overall, data were collected for 57 farms. The AMU derived from the calculation represents the number of days of antimicrobial treatment to which each animal farmed on the establishment may have been potentially exposed during the period considered, in our case one year. The results show high variability between different farms as well as within the same farm over the years: there are indeed years in which no pharmacological treatments were administered and others in which the AMU value dramatically increases. This aspect will be investigated further through the subsequent statistical analysis planned within the project, in which possible associations between AMU and risk and protective factors will be assessed.

(the factors taken into account will include both management and environmental considerations). There are farms (21%) that did not carry out any treatments over all three years considered. An investigation into their characteristics showed that these are farms with a favourable geographical location (altitude, and therefore ideal water temperature, and considerable distance from other farms) and which did not experience relevant bacteriological issues. In these cases, as well, it will be of interest to examine whether the only protective factors are geographical, or whether there are also management-related aspects. According to the EMA's proposed classification of antimicrobials, by examining the data from all the farms, 2022 alone saw a peak in the use of class B antimicrobials; on closer inspection, this was attributable to a single farm that used large quantities of flumequine, having resorted to this class of drugs due to serious fish health issues. In both 2023 and 2024, however, class B accounted for the smallest percentage of antimicrobials used, with classes C and D predominating.

Conclusions

In conclusion, this study highlighted the feasibility of applying a dose-based approach to calculate AMU in trout farms. Preliminary data show considerable variability both between farms and over time, with a non-negligible proportion of farms showing no treatments over extended periods. The subsequent analysis of risk factors will allow for a better understanding and explanation of the observed variability.

Acknowledgment

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OPTIMIZING REFERENCE POPULATIONS FOR LOW-DENSITY SNP IMPUTATION IN ATLANTIC SALMON: IMPACT ON GENOMIC PREDICTIONS

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Introduction

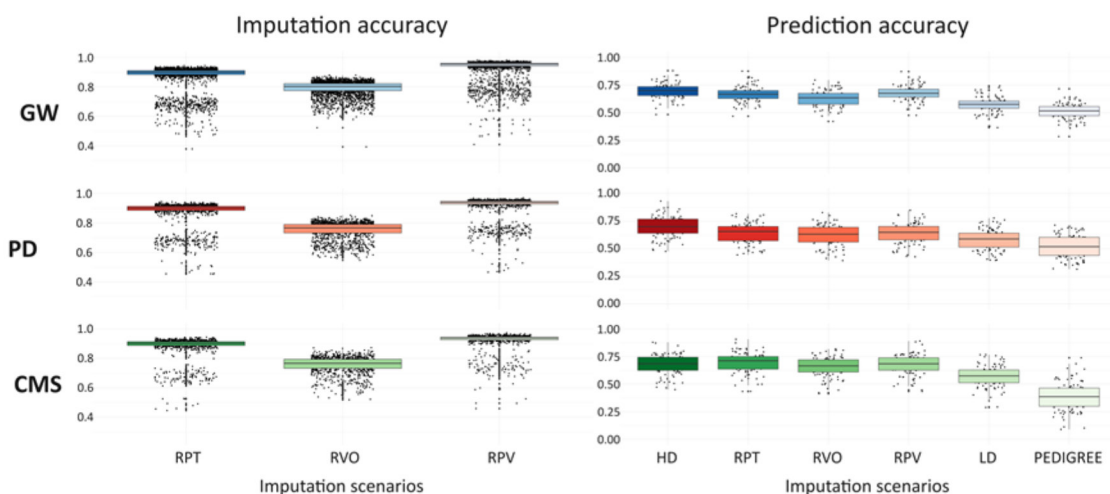
Aquaculture breeding programs rely on a high number of full-sib families with large number of offspring, which has facilitated the uptake of genomic selection (GS). These sib-testing schemes yield high prediction accuracies but require genotyping large training populations and many candidates for effective selection. Lowering genotyping costs could broaden GS use for smaller companies while for larger ones, that have already been implementing GS, reducing the individual cost of genotyping would allow the genotyping of additional candidates, resulting in an increased selection intensity and genetic gain. Recent efforts focused on designing low-density (LD) panels combined with genotype imputation to achieve accuracy similar to medium or high-density panels. In this study we compared scenarios to optimize reference populations for accurate LD-genotype imputation in Atlantic salmon. We investigated how robust or suboptimal imputation affects prediction accuracy for resistance to cardiomyopathy syndrome (CMS), pancreas disease (PD), and growth (GW).

Materials and Methods

Genotypes, pedigree and phenotypes were obtained from salmonids were obtained from MOWI Norway Atlantic salmon breeding programme. All the phenotyped fish and the parents were genotyped with the ThermoFisher Axiom 57K SNP array. After standard quality controls 47,061 SNPs and 1,094 fish for resistance to CMS (gill score), 1,448 for resistance to PD (binary survival) and 2,998 for GW (guttured weight at harvest) were retained. A LD-panel containing 554 pre-established SNPs, was created *in silico* by masking the genotypes of phenotyped fish (LD-target population). The “missing” genotypes of individuals from the LD-target population were imputed to the high density (HD) using the FImpute software. The impact of the design of the HD-genotyped reference population for imputation was tested in three different scenarios with the HD-genotyped reference population composed of i) all the parents (**RPO**: Reference Parents Only), ii) the offspring from the validation set only (1/10th of the offspring; **RVO**: Reference Validation Only) and iii) all parents and the offspring from the validation set (**RPV**: Reference Parents Validation). The accuracy of imputation was estimated as the Pearson’s correlation between the true and the imputed genotype for each individual.

The quality of genomic prediction using pedigree, LD, HD and imputed genotypes was tested in a standard 10-fold cross-validation approach where (genomic) estimated breeding values ([G]EBV) were computed using the BLUPF90 software with a mixed linear animal BLUP model. Accuracy of prediction was computed as the mean over 10 replicates of the Pearson’s correlation coefficient between the (G)EBV and the true phenotypes of fish in the validation group, divided by the square root of the genomic based heritability estimated with the HD-panel.

Figure 1. Accuracy of imputation and predictions for three traits and different imputation scenarios.



(Continued on next page)

Results and discussion

Imputation was overall very accurate (>0.8) for all traits. Scenarios that contained HD-genotyped parents and offspring (RPV and RPV) resulted in the highest accuracy while the scenario with only HD-genotyped offspring (RVO) resulted in a significantly lower imputation accuracy. The genotype of individuals with one or both parents missing from the dataset was badly imputed (correlation <0.5) in scenarios with only parents and the imputation accuracy of those individuals was significantly improved when siblings were added to the HD-reference panel.

For all three traits the accuracy of GEBVs increased significantly with imputation compared to the un-imputed LD-panel. The comparison between the accuracy of prediction obtained from the HD panel and from the imputed panel varied slightly across the traits. For GW, the accuracy of the HD-panel was significantly better than all imputed scenario except for the RPV scenario. For resistance to PD, the predictions with the HD-panel were always significantly more accurate than the predictions obtained with imputed panels. For resistance to CMS, all imputation scenarios were as good as the HD-panel and as good as each other to predict the GEBV of the fish. Those differences may be due to different genetic architecture of the trait with GW being highly polygenic, PD being oligogenic and CMS being monogenic. Interestingly, the difference in the accuracy of imputation obtained between scenarios was not always reflected in the accuracy of genomic prediction. Indeed, the genomic predictions were robust even when genotype imputation was sub-optimal (RVO, Figure 1), although if this scenario resulted in slightly under-dispersed GEBV for GW and CMS.

Conclusion

As aquaculture breeding programmes rely on phenotypes measured on a training population composed of collaterals (full/half-sib) of the selection candidates, an imputation strategy relying on including offspring in an HD-reference panel is particularly suitable for low-cost genomic selection. This strategy could be used to only perform HD-genotyping every two generations, either reducing the cost of genotyping or increasing the selection intensity with minimal decrease in the accuracy of prediction.

Acknowledgment

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EARLYTOX: MOLECULAR EARLY-WARNING SYSTEM FOR HARMFUL ALGAL BLOOMS THREATS IN BIVALVE AQUACULTURE

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Introduction

Aquaculture systems across Europe are increasingly exposed to the impacts of climate change, with rising temperatures, heatwaves, floods, droughts, and extreme storm events already disrupting both freshwater and marine production. Among the most affected sectors is bivalve aquaculture, where the impact of more frequent and intense harmful algal blooms (HABs) is clear. These events pose significant risks to food safety, farm productivity, and coastal economies, particularly when toxin-producing microalgae are present. The EarlyTOX project was implemented to develop a rapid molecular detection approach based on quantitative real-time PCR (qPCR) and digital PCR (dPCR) technologies to detect and quantify toxin-producing microalgae in aquaculture environments, such as *Gymnodinium catenatum*, *Gambierdiscus* spp., and *Ostreopsis* spp..

Results and Discussion

To strengthen earlywarning capacity and improve risk management, molecular detection tools have become essential. qPCR, and dPCR enable rapid, sensitive, and species-specific identification of toxic microalgae in environmental samples. This is especially critical for taxa such as *G. catenatum*, where morphologically similar nontoxic species coexist within the same genus. By targeting genes directly involved in toxin biosynthesis, species-specific primers allow precise discrimination between toxic and nontoxic strains, reducing false positives and supporting current monitoring programs, that are time consuming, labour intensive, and highly dependent on taxonomix expertise, which can delay regulatory decisions. qPCR detection was already achieved for *Gymnodinium catenatum*, *Gambierdiscus* spp. and *Ostreopsis* spp., and dPCR will be optimized to enable highly sensitive and accurate identification and quantification of target species in more complex environmental samples. Results will be compared with monitoring data issued by The Portuguese Institute for the Ocean and Atmosphere (IPMA). This will validate the molecular tool under real-world conditions and evaluate its capacity to support early detection and risk assessment during HAB events. Optimization of detection protocols for other relevant species are ongoing (*Dynophysis acuminata*, *Pseudo-nitzschia*, *Alexandrium*, etc.)

Conclusions

The EarlyTOX project highlights the growing importance of molecular diagnostics in safeguarding aquaculture under climate stress. Integrating advanced detection methods with environmental monitoring provides a robust framework for mitigating HAB-related risks and enhancing the resilience of European aquaculture in a rapidly changing climate.

Acknowledgment

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A NEW FEED SOLUTION IMPROVES GROWTH PERFORMANCE AND MODULATES PHYSIOLOGY OF ATLANTIC SALMON *Salmo salar* DURING SMOLTIFICATION

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Background: Smoltification is a critical physiological transition in Atlantic salmon production, during which fish must adapt from freshwater to seawater while maintaining growth, osmoregulatory capacity, and metabolic stability. This study evaluated whether a novel dietary feed solution, MIXA (MIXSCIENCE, France), could support growth performance and physiological robustness during the freshwater phase and after seawater transfer.

Material and Methods: Atlantic salmon parrs were fed for 12 weeks in a 2 × 2 factorial design combining two basal formulations, a practical smoltification diet and a non-smoltification diet, with or without MIXA at 200 g/ton. The trial included a 6-weeks freshwater phase followed by a 6-weeks seawater phase after direct transfer to full-strength seawater. Growth performance, feed utilisation, survival, plasma biochemical markers, free amino acids, nitrogen-related metabolites, and molecular indicators of gill osmoregulation and intestinal integrity were assessed before and after seawater transfer.

Results: During the freshwater phase, growth performance was mainly driven by the basal formulation: fish fed the smoltification diet showed higher final body weight, weight gain, specific growth rate, protein efficiency ratio, and improved feed conversion compared with fish fed the non-smoltification diet. MIXA induced a modest but significant increase in final body weight, while survival remained 100% in all treatments. In freshwater, MIXA also produced a clear higher metabolic signature, significantly reducing circulating concentrations of most essential amino acids, several non-essential amino acids, and nitrogen-related metabolites including taurine, SAM, SAH, homocysteine, cystathionine, ornithine, GABA, hydroxyproline, and beta-alanine. After seawater transfer, the growth effect of MIXA became more evident, with significant increase in final body weight and weight gain during the seawater phase. The same reduction in plasma free amino acids and nitrogenous metabolites persisted 48 hours after transfer, whereas classical plasma biochemical indicators remained only moderately affected, suggesting good physiological tolerance.

Discussion/Conclusion: The practical smoltification formulation was the main determinant of freshwater performance, whereas MIXA provided an additional growth benefit that was particularly visible after transfer to seawater. The consistent decrease in circulating amino acids and nitrogen-related metabolites suggests that MIXA modulated amino acid turnover, methylation-related pathways, and nitrogen metabolism during smoltification and early seawater acclimation. Overall, these findings support the potential of targeted dietary feed solutions to improve post-transfer growth while maintaining physiological stability in Atlantic salmon undergoing smoltification.

Growth performance throughout the entire duration of the trial (104 of feeding) – Freshwater and Saltwater phases

	PC	PC MIXA	NC	NC MIXA	p-value		
					Formul a (F)	Additiv e (A)	F x A
Survival, %	99.4 ± 1.2	100.0 ± 0.0	99.4 ± 1.2	100.0 ± 0.0	1.000	0.183	1.000
IBW, g	65.4 ± 0.6	64.7 ± 1.5	64.8 ± 1.0	65.1 ± 0.8	0.812	0.624	0.352
FBW, g	176.0 ± 2.6	177.8 ± 2.2	162.8 ± 1.8	173.2 ± 2.3	<0.001	<0.001	0.002
Weight gain/fish, g	110.6 ± 2.5	113.1 ± 2.4	98.0 ± 2.8	108.2 ± 2.6	<0.001	<0.001	0.013
SGR, %/day	0.95 ± 0.01	0.97 ± 0.02	0.89 ± 0.03	0.94 ± 0.02	<0.001	0.002	0.103
FCR	0.97 ± 0.01	0.97 ± 0.01	0.99 ± 0.01	0.99 ± 0.02	0.037	0.951	0.822
FI, %ABW/day	0.85 ± 0.01	0.87 ± 0.03	0.81 ± 0.02	0.86 ± 0.01	0.014	0.007	0.230
PER	2.22 ± 0.03	2.21 ± 0.02	2.18 ± 0.02	2.18 ± 0.06	0.043	0.867	0.764

Values are means ± standard deviation (n=4).

EFFECTS OF DIETARY OREGOSTIM POWDER ON THE HEALTH OF JUVENILE NILE TILAPIA, FOLLOWED BY A *Streptococcus* CHALLENGE

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Introduction

Aquaculture is continuously expanding as a consequence of the demand for healthy and nutritious food, which, along with the global saturation of wild fishery production, has encouraged considerable growth of the sector (Tacon et al., 2025). However, aquaculture is plagued by a plethora of bacterial pathogens, and the rearing of fish at high population densities in aquaculture systems makes them particularly susceptible to disease outbreaks, causing massive mortalities (Behringer et al., 2020). The aquaculture sector has suffered major economic tragedies due to infectious diseases, estimated at around \$6 billion annually (Maezono et al., 2025). The effective control of bacterial diseases, including streptococcosis, through the use of antibiotics has become increasingly challenging in aquaculture as a consequence of antimicrobial resistance (Manage, 2018). Therefore, alternative antimicrobial agents, such as phytogenics, are being sought after to reduce reliance on antibiotics (Bhanja et al., 2023). Oregano essential oil (OEO), has demonstrated an unwavering number of benefits as an aquafeed additive, including well-documented efficacy in improving health, disease resistance, and performance of various aquaculture species (Alagawany et al., 2020). Anpario's Orego-Stim Powder (OSP) is a phytogenic feed additive containing 100% natural, single-source OEO which contains over 100 active compounds, with the primary highly-functional compounds being carvacrol, thymol, *p*-cymene, β -caryophyllene, γ -terpinene, and linalool. These OEO compounds work in synergy to provide a complex mode of action, including antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, and growth-promoting functions (Alagawany et al., 2020), highlighting OSP's potential as an effective nutritional strategy to mitigate disease-related challenges and improve resilience in aquaculture species.

Materials and Methods

This independent feed trial was conducted for 85 days by the State University of Western Paraná in Brazil. A total of 200 juvenile Nile tilapia (*Oreochromis niloticus*), with average initial weight of 50 g, were equally and randomly distributed in 20 conical tanks, each with a usable water volume of 500 L. The trial consisted of five dietary groups with four replications being evaluated; one basal control diet, used as a negative control (NC) during the feeding phase and as a positive control (PC) during the 10day *Streptococcus agalactiae* challenge, and four experimental diets supplemented with either OSP or another phytogenic product at 1 or 3 kg/t of feed. *For the purposes of this abstract/presentation, results from three dietary groups are reported, representing a subset of the full dataset.*

At the end of the trial, zootechnical performance parameters were analysed, including final length, final weight, weight gain, specific growth rate, feed conversion, hepatosomatic index, feed intake, condition factor, and cumulative mortality. The remaining parameters, including digestibility, carcass composition, intestinal enzyme activity (protease, amylase, and lipase), haematology (white blood cell count, lysozyme activity, and antioxidant enzymes), liver and intestinal histology, and intestinal microbiota are still under analysis.

Preliminary Results

The results in Fig. 1 demonstrate a significant effect of dietary groups on cumulative mortality ($p < 0.05$). Among the dietary groups, only OSP 1 kg/t showed a significant effect, indicating an approximate reduction of 86.5% in the risk of death compared with the PC group.

Additionally, greater individual resilience was observed in the OSP 1 kg/t group, with a mean time to death (MTD) of 5.0 ± 1.41 days, higher than that observed in the PC group (MTD = 3.75 ± 0.96 days). Thus, the protective efficacy of OSP 1 kg/t is corroborated by the lower specific mortality rate (SMR) observed in the experiment (SMR = 1.82), indicating a slower progression of the pathogen in this group.

On the other hand, the OSP 3 kg/t diet showed a trend towards statistical significance ($p = 0.067$), with a longer MTD (7.0 ± 2.58 days) and an approximate 60% reduction in mortality relative to the PC.

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THE USE OF HYDROLYSABLE TANNIS IN DIETS FOR PACIFIC WHITE SHRIMP, *Penaeus vannamei*

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Introduction

The rapid expansion of global aquaculture has intensified the need for sustainable, safe, and effective alternatives to antibiotic growth promoters and chemotherapeutics. Increasing concerns over antimicrobial resistance, environmental impact, and residue accumulation in aquatic products have driven research toward natural bioactive compounds with multifunctional benefits.

Plant-derived polyphenolic phytochemicals, particularly hydrolysable tannins (HT), have emerged as promising candidates due to their well-documented antimicrobial, antioxidant, immunoregulating and gut-modulating properties. Natural chestnut extracts, rich in ellagitannins, a specific type of HT, have demonstrated significant potential in terrestrial animal nutrition; however, its application in aquaculture remains relatively underexplored.

In fish and shrimp farming systems, maintaining an optimal growth performance while ensuring disease resistance is critical. Hydrolysable tannins from chestnut extract may enhance feed efficiency, modulate gut microbiota, improve intestinal integrity, support the immune system, and exert antimicrobial activity against pathogenic bacteria commonly affecting aquaculture species.

Materials and Methods

Pathogen-free Pacific white shrimp (*Penaeus vannamei*) were fed four experimental diets during 90 days, after the corresponding acclimation period and placement in the corresponding tanks. The dietary treatments were as follows: Control (commercial feed), T1 (including 1 kg HT/ton of feed), T2 (including 2kg HT/ton of feed), and T3 (including 3 kg HT/ton of feed). After these 90 days, shrimp were collected and sampled for assessment of the growth performance, hepatopancreatic histology and immune-related gene expression.

Results and Discussion

The dietary inclusion of HT resulted in a significantly lower ($p < 0.05$) FCR of shrimp compared to the Control group. In addition, shrimp fed diets T1 and T2 had significantly higher ($p < 0.05$) final body weight (FBW) and specific growth rate (SGR). Shrimp fed diet T1 also had a significantly higher ($p < 0.05$) weight gain (WG) than those fed diet Control. The improvement in the growth performance obtained with the inclusion of HT in the diets was in agreement with the results reported by Novriadi et al. (2023), who also observed a better growth performance in Pacific white shrimp when using similar dietary levels of inclusion of HT.

Regarding the histology of the hepatopancreas, and compared to the Control group, shrimp fed diet T1 had a higher number of vacuoles in the B cells, a lower amount of hemocytes in the intertubular regions, thicker epithelial tissue, presence of R cells, more lipidic reserves and no tissue atrophy. These results were in line with those reported in Pacific white shrimp by Novriadi et al. (2023) with the inclusion of 2 and 3 kg of HT per ton of feed. The hepatopancreas is the main digestive gland of shrimp, and a key indicator of their metabolism (Novriadi et al., 2023). Thus, the observed beneficial effect of the inclusion of 1kg HT per ton of feed on the hepatopancreas, could be indicative of an improved digestive function, when compared to the Control group.

In relation to the immune response, genes related to the immunity, stress tolerance, and antioxidant capacity, were evaluated to assess the effect of the dietary inclusion of HT on the defense system of shrimp. The results indicated a higher ($p < 0.05$) transcriptional response of two out of a total of five genes analysed, these two belonging to the immune and antioxidant responses, in shrimp fed diet T1 compared to those fed diet Control. These results were in agreement with previous studies reporting antioxidant and antimicrobial activity of HT in shrimp, such as that by Huang et al. (2024). Results on the gene expression, as well on the abovementioned parameters, will be further presented and discussed.

To conclude, results from the present study indicated an overall beneficial effect of the dietary inclusion of HT on cultured Pacific white shrimp, suggesting 1kg of HT per ton of feed as the optimal level of inclusion in shrimp diets.

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AQUAPONIC PRODUCTION OF ASIAN SEABASS (*Lates calcarifer*), BASIL (*Ocimum basilicum*) AND MINT (*Mentha sp.*): A COMPARATIVE STUDY OF SYSTEM DESIGN AND WATER QUALITY DYNAMICS

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Introduction

Aquaponics is increasingly recognized as a sustainable food production strategy that integrates aquaculture and hydroponics through internal nutrient recycling, reducing water use and dependence on synthetic fertilizers. This approach is particularly relevant in arid and semi-arid regions where freshwater scarcity and harsh climatic conditions constrain conventional agriculture. However, aquaponic productivity depends not only on system design but also on biological stability, especially the establishment of nitrifying microbial communities responsible for converting toxic ammonia into plant-available nitrate. During early operation, many systems experience instability characterized by pH decline, ammonia accumulation, and reduced fish and plant performance.

Floating raft and media-based systems are among the most common aquaponic configurations. Media-based systems may promote faster microbial colonization because of increased surface area, whereas floating systems are often simpler and easier to manage. Despite these differences, comparative evidence under hot arid conditions remains limited, especially using high-value marine or euryhaline fish species.

Asian seabass (*Lates calcarifer*) is an economically important species with tolerance to a wide salinity range, making it a strong candidate for aquaponics in coastal dry regions. Basil (*Ocimum basilicum*) and mint (*Mentha sp.*) are commercially attractive herbs commonly grown in aquaponics due to rapid growth and moderate nutrient demand.

This study compared floating and media-based aquaponic systems for integrated production of Asian seabass, basil, and mint under greenhouse conditions in Oman. We hypothesized that although system design may influence early-stage water quality dynamics, both systems would converge toward similar performance once nitrification processes were established.

Materials and Methods

The experiment was conducted over 115 days at the aquaponics research facility of Sultan Qaboos University. Six independent aquaponic units were used, each consisting of a fish tank (80 × 40 × 40 cm), sedimentation drum, and plant tank (80 × 40 × 20 cm). Three units were configured as floating raft systems and three as media-based systems using hydrostones.

Asian seabass with an initial mean weight of 163.05 ± 66.95 g were stocked at 7–8 fish per tank to equalize biomass. Fish were fed 2–3 times daily according to feeding response. Basil seedlings were initially planted (8 plants per tank), followed by mint seedlings (3 plants per tank) after basil harvest.

Fish weight and length were measured monthly. Plant height was monitored monthly. Water temperature, dissolved oxygen (DO), and pH were recorded weekly, while ammonia, nitrite, nitrate, phosphate, and silicate were analyzed monthly using standard seawater analytical methods. Differences between treatments were evaluated using independent sample t-tests.

Results

No significant differences were observed between floating and media-based systems for temperature, DO, overall pH, ammonia, nitrate, phosphate, silicate, fish growth, or plant growth.

However, transient instability occurred during Weeks 3–5, when the floating system exhibited significantly lower pH than the media-based system ($p = 0.02$). During the same period, reduced fish feeding activity and slower plant performance were observed.

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Ammonia concentrations were highest during the first two to three months and declined thereafter, indicating progressive establishment of nitrification. Nitrite also decreased over time, while nitrate remained relatively stable in both systems.

Final fish weights were 275 g in floating systems and 281 g in media systems, with identical specific growth rates (1.8% day⁻¹). Final fish lengths were approximately 27 cm in both treatments. Survival rates were 62.5% and 67.3% in floating and media systems, respectively.

Basil and mint showed comparable growth between systems, although slightly greater final plant height was recorded in floating units.

Discussion

The results indicate that system stability rather than physical design was the principal determinant of long-term aquaponic performance. Although media-based systems likely offered greater surface area for microbial attachment during start-up, both configurations converged toward similar water quality and biological outputs once nitrifying communities became established.

The early pH depression and elevated ammonia observed in the floating system are consistent with immature nitrification and insufficient buffering capacity. Nitrification releases hydrogen ions, which can lower pH and temporarily reduce feed intake, nutrient uptake, and fish welfare. Once microbial communities matured, nitrogen transformation improved and both systems achieved comparable functional performance.

Despite similar growth rates, survival remained moderate in both treatments. This suggests that fish survival is more sensitive than growth to short-term environmental stress, particularly fluctuations in pH, temperature, and early nitrogen accumulation. Abrupt seasonal cooling from 30°C to 20°C may also have weakened immunity and increased disease susceptibility.

From an applied perspective, both floating and media-based systems are suitable for aquaponic production in arid regions. However, success depends on rapid system maturation, effective biofiltration, and control of pH and temperature during the initial operational phase.

Conclusion

Both floating and media-based aquaponic systems successfully supported the co-production of Asian seabass, basil, and mint under greenhouse conditions in Oman. No major long-term performance differences were detected between system types. Instead, early-stage environmental instability and delayed nitrification had stronger effects on fish survival and short-term productivity than system configuration itself.

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MECHANICAL DEWATERING BEYOND WATER REMOVAL: A FRACTIONATION TOOL FOR BROWN SEAWEED BIOREFINERY

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Introduction

Mechanical dewatering using a twin-screw press not only reduces biomass moisture content but also facilitates the partitioning of valuable biochemical components between liquid and solid fractions. While mechanical dewatering is commonly applied as a low-energy pre-treatment step, its potential as a fractionation strategy for brown seaweed biorefinery remains largely unexplored. Pressure-driven separation generates a liquid fraction containing soluble compounds and a solid fraction enriched in structural biomass components. In this study, different pre-treatments were combined with twin-screw pressing to evaluate their influence on the distribution of protein-bound amino acids, fucose, and uronic acids in *Alaria esculenta*. The results demonstrated distinct partitioning patterns, with higher concentrations of all three component groups generally retained in the solid fraction, highlighting the potential of mechanical dewatering as an initial fractionation step in cascading seaweed biorefinery. This approach aims to establish mechanical dewatering as a scalable, low-energy entry point for downstream processing, supporting efficient valorization and zero-waste processing.

Methodology

Fresh *Alaria esculenta* was cultivated from Måsskjæra, Norway and harvested on June 2022 (Seaweed Solutions, AS-Norway), this seaweed was subjected to twin-screw pressing without any chemical treatment served as a control (AE.1), washing with freshwater (AE.2), 0.1% KOH treatment (AE.3), or 0.1 M HCl treatment (AE.4) were pressed. Biomass was immersed in each pretreatment solution at a solid-to-liquid ratio of 1:4 for one min before pressing. Dewatering efficiency was assessed through liquid yield, dry matter content of the solid fraction, and solid biomass recovery. The resulting solid and liquid fractions were dried at 50°C and analysed for protein-bound amino acid by HPLC-DAD, fucose by the Dische colorimetric assay, and uronic acids using a modified carbazole method. Whole seaweed biomass served as the baseline reference.

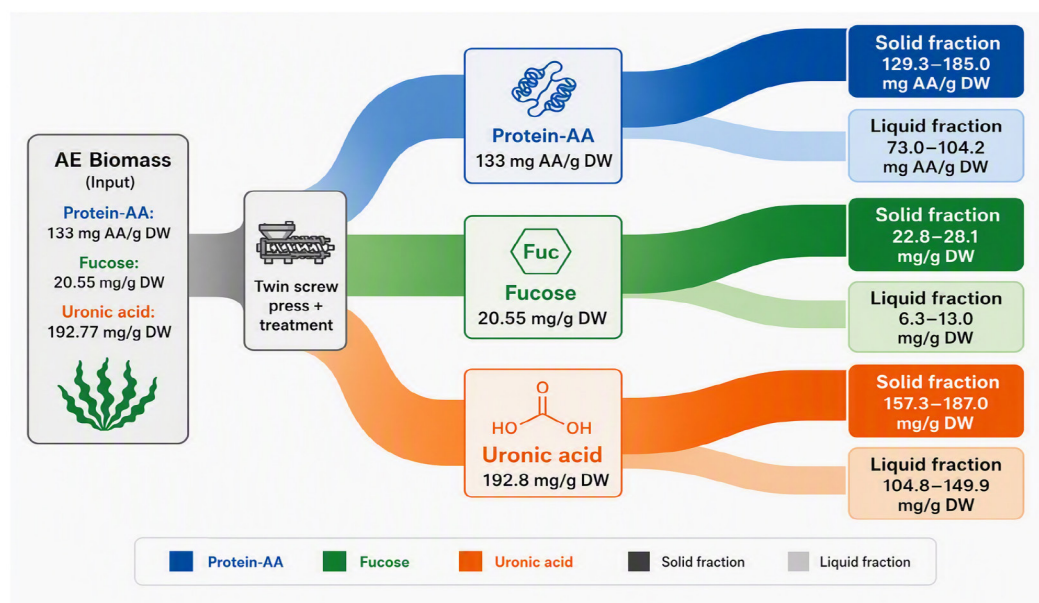


Figure 1 Distribution of protein-bound amino acids, fucose, and uronic acids in solid and liquid fractions of twin-screw-pressed *A. esculenta* (AE1-AE4). Values are expressed as mg g⁻¹ DW.

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Results

Dewatering efficiency varies strongly among treatments, with the highest yield in AE.2 (86.95%), followed by AE.3 (66.92%), AE.4 (54.07%), and AE.1 (51.98%). With the solid fraction had the highest DM% in AE.4 (35.40%), while AE.2 showed the lowest solid recovery (37.75%), indicating greater transfer of material into the liquid phase. Processing loss was highest in control sample (AE.1 = 20.11%) and lowest in AE.3 and AE.4 (7.22% and 8.02%), suggesting better mass retention by mild acid/alkali treatment.

Protein

Twin-screw pressing retained protein-bound amino acids mainly in the solid fraction by the given treatment in the range of 129–185 mg AA/g DW, with HCl pretreatment (AE.4) achieving the highest retention/enrichment, compared to baseline value of 133 mg AA/g DW. Essential amino acids (leucine, valine, isoleucine, and phenylalanine) were enriched after pressing, this confirms that majority of essential amino acids were in the pressed solid fraction, whereas liquid fractions contained polar amino acids in the range of 73–104.2 mg/g DW, indicating that these amino acids can be recovered by further processing. Overall, pressing enabled selective fractionation of protein-rich solid and soluble amino acid streams for biorefinery applications.

Fucose and uronic acid

Mechanical dewatering influenced the partitioning of both fucose- and uronic acid-containing polysaccharides in *A. esculenta*, but with different retention patterns. Uronic acid remained highly concentrated in the solid fractions (157–187 mg/g DW), representing 82–97% of the raw biomass level, indicating retention of alginate-bound polymers, whereas liquid fraction has uronic acid in the range of 104.8–149.9 mg/g DW, this confirms soluble sugars moved to the liquid fraction. In comparison, fucose showed greater enrichment in the solid fractions (22.8–28.1 mg/g DW; 111–137% of raw biomass level), suggesting removal of salt concentration enriched fucoidan availability which reflects on the fucose content. The liquid fractions contained considerably lower fucose concentrations (6.3–13.0 mg/g DW) compared to uronic acid (104.8–149.9 mg/g DW). Overall, screw press dewatering acted as a selective fractionation step, retaining fucose-rich fucoidans and alginate-related uronic acid in the solid fraction while generating a liquid fraction enriched in soluble compounds.

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.

DEWATERING EFFICIENCY OF HYDRAULIC PRESSING OF *Ascophyllum nodosum* BIOMASS

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Introduction

Brown seaweed *A. nodosum* has been widely used for feed and biostimulant purposes for many years. However, due to its *high-water content* (ranging >80% MC) it presents challenges like increasing costs of transportation and storage for downstream processing. Therefore, an effective dewatering step is essential to reduce the moisture content, concentrate the biomass and improve handling and processing efficiency. Mechanical dewatering is commonly applied as a pretreatment step in biorefining as it removes readily mobile water and reduces the moisture available for microbial deterioration during handling and storage. However, the efficiency of mechanical dewatering can be limited by the strong water-holding capacity of seaweed tissues and cell-wall components, resulting in substantial residual moisture in the pressed biomass. In this context, this study evaluates calcium chloride (CaCl_2) and magnesium chloride (MgCl_2) as pretreatment agents prior to hydraulic pressing of *A. nodosum*, assessing their effects on dewatering efficiency and the partitioning of protein-bound amino acids between the liquid and solid fractions.

Methodology

Ascophyllum nodosum was harvested at Mørkvedbukta (Bodø) in Norway in February 2022 during low tide and cleaned with seawater. Firstly, *A. nodosum* was minced using a meat mincer to reduce the particle size to ~1 mm, during this mincing process 5% (w/w) CaCl_2 was added to one batch (AN. CaCl_2), and 5% (w/w) MgCl_2 was added to another (AN. MgCl_2) and a third untreated batch served as a control. The salts were incorporated during the mincing process for uniform distribution throughout the biomass. These were then pressed using a hydraulic press (~0.138 MPa), and the resulting liquid and solid fractions were collected to evaluate dewatering efficiency (%). The latter was defined as the proportion of water recovered in the liquid fraction relative to the initial water content of the fresh biomass. The initial water content was calculated from the fresh weight and dry matter (DM %), while the water recovered in the liquid fraction was determined by subtracting the dry matter content from its total mass. The fractions were dried and further analysed for mineral distribution by ICP-OES and total protein-bound amino acid composition by HPLC-DAD.

Results

Dewatering efficiency

The dewatering efficiency (DE %) of *A. nodosum* differed considerably between AN. CaCl_2 and AN. MgCl_2 treatments, AN. CaCl_2 removed 36.15% of water of, while the AN. MgCl_2 treatment only removed 27.84%, and only negligible amounts of water were removed from untreated *A. nodosum*. The solid fraction obtained after AN. CaCl_2 treatment exhibited a higher dry matter content (38.96 DM%) compared to the AN. MgCl_2 treatment (36.52 DM%). These results demonstrated that AN. CaCl_2 created a more concentrated solid fraction that could be advantageous for downstream processing and biomass preservation (Figure 1). The high liquid ash content indicates substantial partitioning of inorganic components/salts into the liquid fraction, with a stronger effect observed for AN. CaCl_2 . Overall, the CaCl_2 pretreatment enhanced water removal more effectively, while both treatments retained most of the biomass dry matter in the solid fraction.

Total protein-bound amino acids distribution

The control solid fraction had the highest total protein-bound amino acid concentration (53.62 ± 4.37 mg AA/g DW) (Figure 2). Whereas, salt pretreatment reduced the amino acid concentration in the solid fraction to 36.07 ± 1.04 mg AA/g DW with AN. MgCl_2 and 34.95 ± 1.69 mg AA/g DW with AN. CaCl_2 . Relative to the control, this represents a decrease of 32.7% with MgCl_2 and 34.8% with CaCl_2 .

Conclusion

Overall, salt-assisted dewatering improved biomass dewatering while enabling selective fractionation of nutrient between the liquid and solid streams with better retention of protein-bound amino acids. suitable for downstream biorefinery applications, while both treatments generated a inorganic components in the liquid fraction, the solid biomass enrichment with essential compounds for further valorization.

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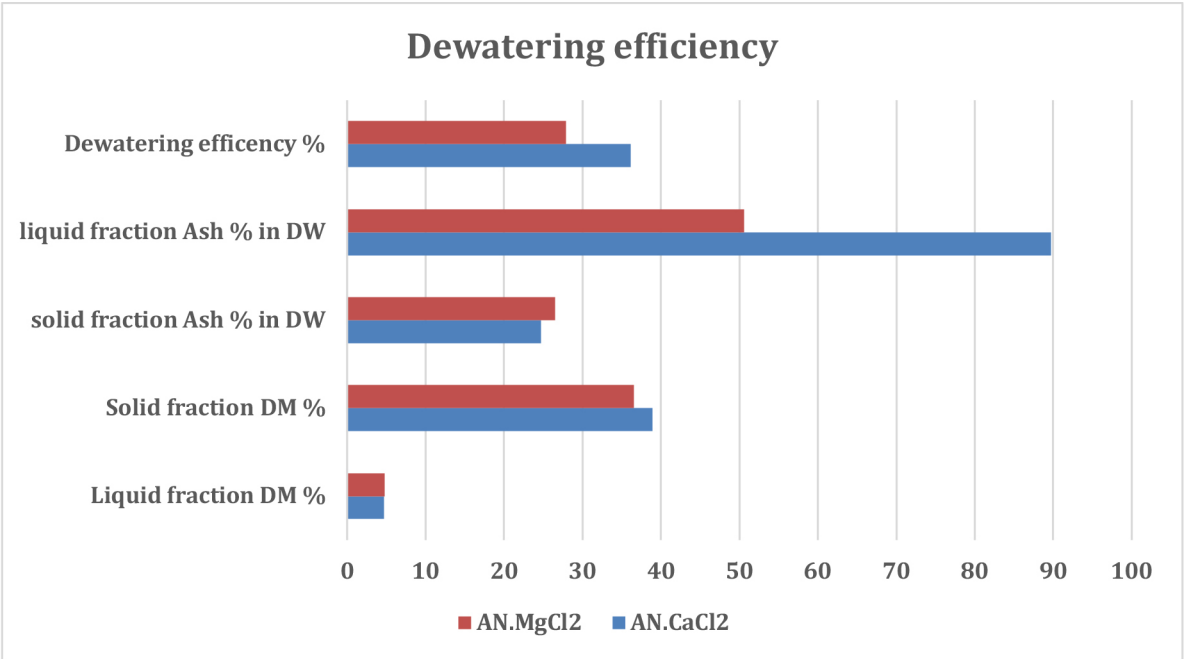


Figure 1: Dewatering efficiency of hydraulic pressing of *A.nodosum* under CaCl_2 and MgCl_2 treatments.

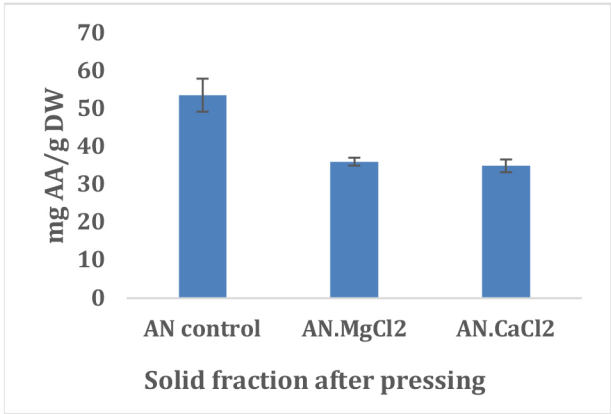


Figure 2: Total-Protein-bound amino acids retained in solid fraction compared to untreated control (in mg AA/g DW)

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.

NET ENERGY EVALUATION SYSTEM FOR CARNIVOROUS FISH SPECIES

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Aquaculture production has risen steadily over the past 40 years, surpassing capture fisheries around 2022 (FAO, 2024). Sustaining this growth requires more aquafeed and improved, sustainable formulations. Due to rising costs and limited supplies of fish meal and fish oil, feeds are increasingly shifting to plant-based and novel ingredients—such as insect meals, single-cell proteins, and industrial by-products—supporting more circular and sustainable production, to keep up with the demand. Additionally, this increases the variability in ingredient and macronutrient composition of new aquafeeds. Aquaculture is characterized by a high species diversity with specific nutritional requirements, thereby highlighting the need for accurate species-specific fish feed evaluation systems. Providing a more accurate energy-based formulation results in a more efficient diet by better predicting energy retention and growth performance. Currently, dietary energy evaluation and feed formulation for fish is established on a digestible energy (DE) basis. This system is based on the DE obtained from the diet using the general efficiency (fraction from 0 to 1) of utilization of the dietary energy for retaining energy (RE), also fulfilling basic metabolism of the animal. This system projects a linear relationship between the RE obtained from a certain DE intake. Estimating an equation (i.e., $RE = \mu + \beta DE$), where the slope (β or KgDE) is the utilization efficiency of DE for energy gain, and the minimal metabolism (μ) known as the fasting heat production. This system does not make a distinction between the source of energy (protein, fat, and carbohydrate) in the diets, thus assumes one generalized efficiency. Nevertheless, recent studies have demonstrated this to not be true, obtaining different efficiencies of energy use between diets differing in macronutrient composition. To consider this variability in the utilization of the different macronutrients, the KgDE is divided into three types of digestible nutrient intake; protein (dCP), fat (dFat), and carbohydrates (dCarb), estimating how efficiently the animal can obtain energy from each one of them. This is known as the net energy (NE) system, already being used in cows, pigs, and poultry, and currently being developed for fish. In this method RE is a function of the three separate macronutrients (i.e., $RE = \mu + \beta_1 dCP + \beta_2 dFat + \beta_3 dCarb$) where the different slopes (β_1 , β_2 , and β_3) are coefficients of the utilization (KgNE) of each digestible macronutrient intake individually (KgNE;dCP, KgNE;dFat, and KgNE;dCarb) for energy gain. Those coefficients can be used to calculate the efficiency of utilization by dividing the coefficient (β_1 , β_2 , and β_3) by the energetic value of each macronutrient; 24.6, 39.5 and 17.2 kJ for protein, fat and carbohydrates, respectively. This approach allows to obtain different recommended NE intakes based on the macronutrient variation of the diets, with species-specific formulas of RE. Recently, NE equations for several fish species have been estimated: rainbow trout, Nile tilapia, barramundi, snakehead, among others (Phan, 2022; Schrama et al., 2018). A relatively low variability in efficiencies was detected in dCP (47% to 64%) and dFat efficiencies (78% to 93%). However, the highest variability occurred in the dCarb fraction, with efficiencies ranging from 5 to 70%, being relatively low in carnivorous (high trophic) fishes. This variation remarks the species-specific nature of the nutrient utilization, supporting the need for species-specific formulations, as is currently being addressed. Recently, new NE equations for relevant carnivorous fish species of production interest such as: Atlantic salmon (*Salmo salar*), European Seabass (*Dicentrarchus labrax*), and Yellowtail Kingfish (*Seriola lalandi*) have been estimated. New estimations of RE have been made using separate coefficients for dCP, dFat and dCarb. In addition, this coefficients also allow to calculate the utilization efficiency of energy in the form of protein, fat, and carbohydrate (those will be presented). This approach enhances the accuracy of estimating retained energy. This enables to make a more accurate prediction in new aquafeeds with high variability of macronutrient composition; hereby supporting the shift to novel ingredients.

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THE ECONOMICS OF RECIRCULATING AQUACULTURE SYSTEMS: A SYSTEMATIC REVIEW AND SYNTHESIS OF COST STRUCTURES, PROFITABILITY, AND PERFORMANCE DRIVERS

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Introduction

Global food systems increasingly require production models that improve resource efficiency while reducing environmental impacts. In this context, aquaculture has become one of the fastest-growing food production sectors and is expected to play a key role in future food security.

Intensive aquaculture systems such as Recirculating Aquaculture Systems (RAS) have gained attention due to their ability to reduce water use, recycle nutrients, and sustain high production densities under controlled conditions. However, despite their environmental advantages, their long-term economic sustainability remains uncertain (FAO, 2024; Palm et al., 2018; Verma et al., 2023). An integrated agri-aquaculture system, is an interdisciplinary approach that addresses the water-food-energy nexus of United Nations' sustainable development goal and increasing demands of global population at minimal ecological cost. Embracing the 'circular bio-based economy' concept, aquaponics has exhibited immense potential for reduced resource utilization, discharge mitigation, nutrient, energy, wastewater recycling, and production of highly nutritious food within the system. Aquaponics has attained increased public interest in recent years in urban areas, in line with the trend towards greater productivity with limited resources, driving it from small-scale operation to brink of commercialization. The holistic analysis of scientific articles on aquaponics has perceived the transition of research trends from optimization of system performance (component ratio, hydraulic loading rate, etc.). Several commercial ventures have faced financial and operational difficulties linked to high capital investment, energy dependence, biological risk, and management complexity (Engle, 2023; FS, 2025; SalmonBusiness, 2024).

Existing studies show that profitability is highly sensitive to factors such as electricity prices, labor costs, stocking density, survival rates, and market access. Nevertheless, economic evidence remains fragmented and difficult to compare due to differences in scale, technology, and performance indicators. Most research emphasizes technical efficiency rather than systematic assessments of economic risk, scale effects, and long-term viability, creating uncertainty for investors, policymakers, and producers (Babatunde et al., 2023; Benjamin et al., 2020; Blidariu & Grozea, 2011).

Materials and Methods

This study applies a systematic literature review (SLR) to evaluate the economic and financial performance of RAS. A structured review process was used, including database searches, screening, eligibility assessment, and qualitative synthesis of findings. Peer-reviewed studies (n=74) published between 2010 and 2025 were collected primarily from Scopus and complemented with Google Scholar searches. The review focused on publications addressing economic performance, profitability, investment, and techno-economic aspects of RAS, while purely technical studies without economic analysis were excluded. After screening and selection, relevant studies were analysed to identify recurring economic drivers, cost structures, profitability patterns, methodological gaps and key challenges affecting the long-term viability of RAS.

Results and Discussion

The reviewed literature on RAS and aquaponic systems has expanded considerably since 2010, particularly across Asia, Europe, and North America, reflecting the growing interest in intensive and resource-efficient food-production systems. However, despite this increasing body of research, substantial methodological and economic heterogeneity remains across studies. For example, energy and feed expenditures consistently emerged as the most recurrent operational cost drivers, yet their reported contributions to total operating costs varied widely depending on system configuration, species, scale, and analytical assumptions. Several studies highlighted that energy-intensive infrastructure associated with pumping, oxygenation, and thermal regulation may represent a major barrier to economic viability, while feed costs alone accounted for approximately 24–70% of operating expenses across different production systems.

At the same time, the literature revealed persistent gaps in standardized economic reporting, including the lack of disaggregated energy-consumption data, inconsistent functional units, and limited treatment of stochastic risks such as energy-price volatility and infrastructure dependency. These findings are particularly relevant for industry stakeholders and policymakers, as they highlight the need for more comparable, risk-oriented, and scale-sensitive techno-economic assessments to support investment decisions and the sustainable expansion of RAS technologies.

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Acknowledgments

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PREVALENCE OF BUCEPHALID TREMATODE INFECTION IN THE EUROPEAN FLAT OYSTER (*Ostrea edulis*) ALONG THE CROATIAN COAST

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Introduction

The cultivation of the European flat oyster, *Ostrea edulis* (Linnaeus, 1758), in Croatia has a tradition that goes back centuries, and it is still the most important farmed bivalve in the country. When in the early 1970s and 1980s two diseases caused by protozoa, *Marteilia refringens* and *Bonamia ostreae*, began spreading and drastically reducing *O. edulis* production in almost all traditional European farming areas, Croatian populations were not affected, so this species remains the only cultivated oyster in the country. Since the farming of *O. edulis* relies on the collection of spat from the natural environment, it is of the utmost importance to ensure a healthy broodstock. Bucephalid trematode larval stages are known to be common parasites of commercially important bivalve species. They invade the interstitial connective tissues and obstruct digestive gland tubules, thereby affecting food absorption and destroying the surrounding gonadal tissue. Depending on how intense the infection is, these trematodes can completely destroy the gonads by replacing the host tissues, severely affecting the reproductive ability and survival of both, wild and farmed oyster broodstock (Gadelha, et al., 2025). The first case of bucephalid infection of *O. edulis* in Croatia was reported in 2009 in Mali Ston Bay, the largest oyster-producing area (Gavrilović et al., 2009). Keeping in mind the economic importance of oysters, the aim of this study was to examine the presence of digenean parasites in the digestive gland and gonadal tissue of *O. edulis* in wild and farmed populations along the Croatian coast.

Materials and Methods

At the beginning of May 2025, *O. edulis* specimens were collected randomly at five sampling locations (Figure 1). Samples were taken at three farming locations (1 - Mali Ston Bay, 3 - Krka River estuary near Šibenik, 5 - Medulin Bay), while in the Neretva River estuary (2) and near the island of Vrgada in Pakoštane area (4), samples were taken from wild populations. At each location 20 oysters were sampled, except for locations 1 (22 specimens) and 5 (29 specimens). For the purpose of histological examination, visceral parts of oyster soft tissue were fixed in Davidson's fixative. After routine histological processing, material embedded in paraffin was serially sectioned at 5 µm. Sections were stained with hematoxylin and eosin (H&E) and studied by light microscopy. The infection was evaluated in histological sections, and its prevalence was calculated at each sampled location.

Results and discussion

The observed prevalence of parasites varied depending on location (Figure 2). In wild oysters from Vrgada Island, no infection was detected. The highest prevalence of parasites was found in farmed oysters in the Medulin Bay (44%), followed by the Neretva Estuary (40%). In the Mali Ston Bay, the prevalence was 18%. If the results from the Mali Ston Bay are compared with the results of Gavrilović et al (2009) for the same area, it could be assumed that the prevalence has not changed significantly. These authors found temporal variations in the level of infection, where the monthly prevalence ranged from 0-20%, with the maximum in November. During the winter months, from December to March, no infection was detected, which could be due to mortality caused by starvation from severe infection. These results showed that bucephalid trematode infection occurs in *O. edulis* along the Croatian coast, raising concerns about potential effects on broodstock reproduction and survival. In heavily infected European flat oysters it can be reflected in the lack of recruitment. Considering the economic importance of this species, it would be advisable to further investigate the exact bucephalid species using molecular methods and identify its final host.

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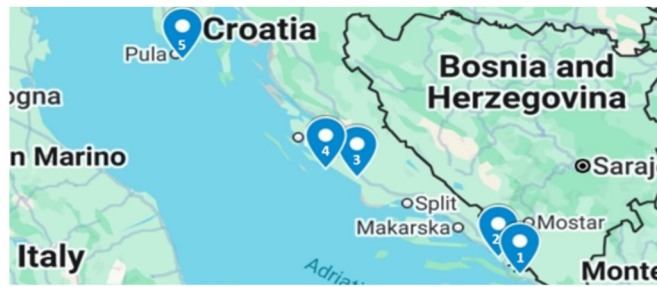


Figure 1. *O. edulis* sampling locations along the Croatian coast

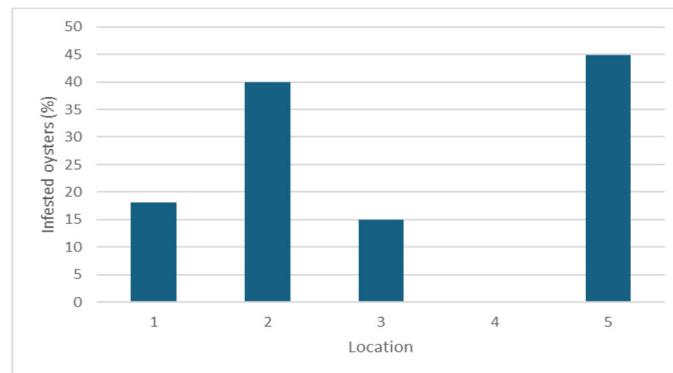


Figure 2. Bucephalid infection prevalence in *O. edulis* from each sampling location

Acknowledgment

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EVALUATION OF LOW OPPORTUNITY COST BIOMASS FEED FOR LOW-TROPHIC-LEVEL FINFISH AQUACULTURE: A STUDY WITH NILE TILAPIA

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Introduction

Aquaculture increasingly relies on refined feed ingredients that may compete with resources suitable for human food production. Greater use of low-opportunity-cost biomass (LOCB), including processing by-products and other underutilised resources, could improve feed circularity (Verreth et al., 2023), provided fish performance and edible-product quality are maintained without unacceptable trade-offs in nutrient utilisation or waste management. Nile tilapia (*Oreochromis niloticus*) is well suited to testing this concept. We evaluated whether increasing LOCB use could reduce feed-food competition while maintaining production performance and edible-product nutritional quality.

Materials and Methods

Two diets were formulated: a conventional control (CON) based predominantly on refined ingredients and a treatment (TREAT) with increased locally available LOCB. Both targeted comparable predicted digestible protein and energy; TREAT contained higher crude nutrient concentrations to compensate for expected lower digestibility of selected LOCB ingredients. Both diets met essential amino acid requirements and were produced as floating extruded pellets.

A 90-day feeding trial used mixed-sex Nile tilapia (~144 g initial body weight) in three tanks per feeding system. CON and TREAT were maintained in separate ~4,500-L recirculating aquaculture systems and fed three times daily at 2% biomass. Growth, feed utilisation, survival and fillet nutritional quality were evaluated. Because diet was confounded with RAS, comparisons were exploratory and based on tank means. A separate replicated trial (four tanks per diet) determined apparent digestibility and faecal particle integrity. Resource-use indicators quantified LOCB and food-competing feed/protein inputs and conversion into edible fish nutrients.

Results

LOCB inclusion increased from 21.5% in CON to 52.4% in TREAT, while food-competing ingredients decreased from 76.5% to 44.6%. FCR was comparable (1.74 ± 0.03 vs. 1.76 ± 0.04 ; $p = 0.410$), while exploratory comparisons indicated higher weight gain (202.7 ± 5.0 vs. 216.0 ± 3.8 g fish⁻¹; $p = 0.024$) and SGR (0.976 ± 0.015 vs. $1.015 \pm 0.008\%$ day⁻¹; $p = 0.030$) in TREAT; survival exceeded 97% in both systems. Food-competing feed input decreased by 41% (1.331 to 0.785 kg kg⁻¹ biomass gain) and food-competing protein input by 54%. With similar net edible protein gain, TREAT converted food-competing dietary protein into edible fish protein approximately 2.2-fold more efficiently. Final fillet yield (36.31 ± 2.28 vs. $36.47 \pm 1.39\%$), proximate and amino-acid composition, and EPA+DHA concentration (0.468 ± 0.018 vs. 0.446 ± 0.036 mg g⁻¹ wet weight; $p = 0.423$) were comparable. TREAT showed lower apparent protein digestibility (82.1 vs. 90.1%) and more disintegrable faeces (54.0 ± 11.1 vs. $25.7 \pm 4.4\%$; $p < 0.01$), with fewer easily removable solids (46.0 ± 11.1 vs. $74.3 \pm 4.4\%$; $p < 0.01$).

Discussion

Increasing LOCB use substantially reduced dependence on food-competing biomass and protein without an evident penalty in feed conversion, survival, fillet yield or nutritional quality. This advantage became clearer when feed efficiency was evaluated according to resource opportunity cost rather than total feed or protein input. However, lower protein digestibility and poorer faecal particle integrity reveal a trade-off between food-system circularity and nutrient and solids management in intensive RAS. LOCB-based formulation is therefore promising, while digestibility and physical waste quality remain important optimisation targets.

Acknowledgement

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A WATER-SAVING APPROACH TO CRAYFISH AQUACULTURE: COMBINED EFFECTS OF LIMITED WATER EXCHANGE AND PROBIOTICS

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Introduction

Freshwater scarcity is becoming a major operational constraint for inland aquaculture and sustainable production planning (FAO, 2022). The red swamp crayfish (*Procambarus clarkii*) is a high-value freshwater decapod, but intensive culture commonly relies on regular water renewal to control nitrogenous wastes and maintain microbial stability (Boyd & Hanson, 2010). Limited water exchange can reduce water consumption, yet its interaction with probiotic supplementation and host-associated microbiota remains insufficiently understood. Previous work indicates that crayfish gut microbiota is closely linked with growth stage, metabolism and health status (Chen et al., 2021; Liu et al., 2023). This study evaluated whether low-volume weekly water exchange combined with probiotics can sustain growth, innate immune response and gut microbial balance in juvenile *P. clarkii*.

Materials and Methods

Juvenile crayfish were reared for 84 days in a 3 × 2 factorial design combining three weekly water exchange rates (1%, 3% and 6% of tank volume) with or without probiotic supplementation. Six treatments were tested in triplicate: 1WE, 1WEP, 3WE, 3WEP, 6WE and 6WEP. Each 40 L tank was stocked with 12 juveniles and maintained under continuous aeration. The probiotic mixture, containing *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus salivarius* and *Bacillus subtilis* at 1 × 10⁹ CFU mL⁻¹, was applied at 1 mL 100 L⁻¹, consistent with the use of lactic acid bacteria and *Bacillus* spp. in crustacean culture (Foysal et al., 2020). Crayfish were fed a 35% crude-protein diet at 3% biomass day⁻¹. Water quality was routinely monitored following standard aquaculture principles (Boyd & Hanson, 2010). Growth performance, survival, feed utilization, total haemocyte count, and gut microbiota were evaluated. Midgut samples were pooled by replicate for 16S ribosomal ribonucleic acid sequencing, and phylum-level microbial composition and diversity indices were calculated (Chen et al., 2021; Liu et al., 2023).

Results

Water quality remained within suitable ranges for juvenile crayfish. Probiotic-supplemented groups showed lower total ammonia nitrogen and nitrite concentrations, especially under 3% and 6% water exchange. The best overall performance was obtained in 3WEP, with the highest final body weight (8.0 ± 0.5 g), weight gain (156.4 ± 5.2%), specific growth rate (1.42 ± 0.03% day⁻¹), survival (95.8 ± 2.1%) and total haemocyte count (7.2 ± 0.4 × 10⁶ cells mL⁻¹). Probiotic supplementation significantly improved all measured growth parameters at 3% exchange, while its effect was weaker at 1% and not significant at 6%. Gut microbiota was dominated by Proteobacteria, Bacteroidota, Firmicutes and Actinobacteriota. The 3WEP group showed the lowest Proteobacteria proportion (42%), higher Bacteroidota (28%) and Firmicutes (20%), and the highest Shannon, Simpson and Pielou evenness values.

Discussion

These findings suggest that 3% weekly water exchange provided a favourable balance between waste dilution and microbial stability, supporting both water-quality control and reduced freshwater demand (FAO, 2022; Boyd & Hanson, 2010). Under this condition, probiotics appeared to improve feed utilization, innate immune readiness and gut microbial balance, consistent with previous reports on probiotic effects in freshwater crustaceans (Foysal et al., 2020). The lower Proteobacteria and higher Bacteroidota and Firmicutes in 3WEP indicate a more balanced gut profile, although phylum-level data should be interpreted cautiously and supported by future genus-level or metagenomic analyses (Chen et al., 2021; Liu et al., 2023). Overall, the 3WEP strategy reduced water use by approximately 97% while maintaining high survival and improving performance, suggesting a promising water-saving approach for juvenile crayfish culture. Commercial-scale validation is still needed before farm-level recommendations are made.

(Continued on next page)

Acknowledgment

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EFFECTS OF ASTAXANTHIN AND SPIRULINA (*Arthrospira platensis*) ON GROWTH PERFORMANCE AND SKIN COLORATION OF MOLLY FISH (*Poecilia sphenops*)

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Introduction

The molly fish (*Poecilia sphenops*) is one of the important ornamental fish species that, due to its diverse coloration, high adaptability to environmental conditions, and ease of reproduction, holds a special position in the aquarium industry. In the culture of ornamental fish, in addition to proper growth, improvement of body coloration is also of great importance, and nutrition plays a fundamental role in achieving these objectives. The use of natural pigment sources such as astaxanthin and the microalga Spirulina in the diet can lead to improvement in growth performance and coloration in fish. The aim of the present study was to investigate the effects of astaxanthin and Spirulina supplements on growth indices and coloration characteristics of molly fish.

Materials and methods

In this study, 90 molly fish with an initial mean weight of 1.5 ± 0.1 g were allocated to three treatments including a control treatment (without supplement), an astaxanthin treatment (10 μ g astaxanthin per 10 g dry weight of feed), and a Spirulina treatment (*Arthrospira platensis*) (0.5 g Spirulina powder per 9.5 g feed), with three replicates and ten fish per replicate. The fish were cultured for a 40-day experimental period and were fed once daily. Fish were maintained in glass aquaria under controlled conditions at a temperature of 27 ± 0.5 °C. Growth indices including final weight, weight gain, total length, length increase, mean total weight gain, daily weight gain, specific growth rate, and feed conversion ratio were calculated. In addition, colorimetric indices were measured using a spectrophotometer based on the CIELAB color system including L*, a*, and b*, and derived color indices were also calculated. The data of the treatments and replicates of the three experimental groups were analyzed using multivariate statistical analysis in PRIMER V6 software and nMDS analysis (non-metric multidimensional scaling).

Results

The results showed that the experimental groups were clearly separated and different from each other based on the set of growth indices and color indices (2D Stress = 0.000). The Spirulina-supplemented treatment showed the greatest effect on growth indices and led to increases in final weight, length increase, mean weight gain, specific growth rate, and improvement in feed conversion ratio compared with the control treatment. In contrast, the astaxanthin treatment had the greatest effect on color indices and caused significant increases in redness (a*), yellowness (b*), and color brightness (stress < 0.05).

Discussion

In conclusion, dietary supplementation significantly affected both growth performance and coloration in molly fish. Spirulina improved growth-related indices, including weight gain, length increase, and feed conversion efficiency. In contrast, astaxanthin had a stronger effect on pigmentation and enhanced color intensity. Multivariate analysis confirmed clear differences among treatments based on growth and color indices. These findings indicate that Spirulina and astaxanthin can be effective dietary supplements in molly fish culture.

A LOW-COST UNMANNED SURFACE VEHICLE FOR STATISTICALLY-INDEPENDENT MULTI-DEPTH WATER SAMPLING IN AQUACULTURE PONDS

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Introduction

Sustainable pond aquaculture depends on water-quality data that is representative both laterally and across the vertical profile because dissolved-oxygen, temperature, and ammonia gradients drive fish welfare, feed conversion and disease risk, and these gradients sharpen under Aquaculture in Global Change conditions. Manual grab sampling caps the number of stations, the sampling rate and the consistency of QA/QC protocols, while commercial autonomous samplers (Teledyne ISCO, Hach Sigma SD900, OceanAlpha ESM30) cost roughly US\$11,000–110,000 and earlier academic low-cost USVs (e.g. Madeo et al., 2020; Jo et al., 2019) follow fixed-grid or manually-piloted protocols and do not enforce a statistical-independence criterion between samples. We address this gap with an unmanned surface vehicle (USV) costing less than US\$5,000. Concrete contributions: (i) an integrated low-cost USV platform (catamaran hull, ESP32 control, peristaltic pump, 7-position bottle carousel, stepper-winch depth probe with end-of-tube weight; Fig. 1) demonstrated end-to-end in a half-Olympic pond; (ii) a correlated random walk (CRW) “bump-and-turn” navigation policy with a $\tau_{\min}$ -gated sampling rule enforcing a minimum separation $D_{\min} = 2l$ without on-board GPS; (iii) an overhead ArUco optical-tracking pipeline that reconstructs each sample position post-hoc as a spatial-statistics ground truth; (iv) a paired simulator/field validation including a documented wall-stuck failure mode and its planned firmware fix (Codling et al., 2008; ISO 5667-1; USGS-NFM). The platform targets the small-to-medium land-based ponds and raceways that dominate Mediterranean and inland aquaculture.

Materials and Methods

The USV is a catamaran with twin PVC pontoons and a 3D-printed deck (1.0 m × 0.55 m, 4.5 kg), driven by twin DC motors with air propellers and supervised by an ESP32 microcontroller that delegates the sampling sequence to an Arduino UNO over UART. Multi-depth sampling uses a peristaltic pump (5.0–5.2 mL s⁻¹; 100 mL fill in 19.3–20.5 s), a drain/bottle selector valve, and a 7-position carousel; depth is set by winch position, eliminating the need for a pressure transducer. Navigation is a CRW with a “bump-and-turn” rule: front micro-switches with 20–40 ms software debounce trigger a short reverse and a random turn (30°–90°), yielding directional persistence in a confined geometry. A minimum separation $D_{\min} = 2l$ is enforced as a design constraint; l is a conservative engineering design value ($l = 2$ m, giving $D_{\min} = 4.0$ m), with an in-situ semivariogram refinement on the validation roadmap. The constraint becomes a real-time decision rule $\tau_{\min} \approx D_{\min} / v \approx 20$ s for $v = 0.20$ m s⁻¹, so the platform enforces sample separation without on-board GPS — appropriate for covered or indoor ponds and raceways where GPS is unreliable. QA/QC is enforced via a purge ratio $t_{\text{flush}} = k \cdot V_{\text{dead}} / Q$ with $k \in [2,3]$ per ISO 5667-1 and USGS-NFM (Chapter A4). Independence is verified post-hoc with Moran’s I on ArUco-reconstructed sample positions; ground truth comes from a single overhead 1920×1080 wide-FOV (90°–100°) camera at 7 m with 22–25 cm ArUco markers (≥ 70 px at the far edge).

Results and Discussion

A 40-min CRW simulation on a 12.5 × 20 m pond (matching the field pool) reached 93 % coverage with 10 sample stops and a near-uniform spatial distribution ($I_X = +0.04$, $I_Y = +0.10$; $R = 1.59$), confirming the design behaviour. The field trial (Fig. 2) captured 147 ArUco positions along a continuous trajectory and 14 sample stops on Pool-2; mean inter-sample distance was 4.16 m and the maximum 11.1 m. Of 91 all-pair distances, 16 (18 %) fell below $D_{\min}$ and 5 of 13 consecutive pairs reached $D_{\min}$ (Fig. 2c). Sample volume was consistent (mean fill 25.9 mL across 7 bottles vs. a 27 mL target, ~96 %); blank–tracer–blank cross-contamination stayed below the 1 % carry-over threshold, consistent with ISO 5667-1 and USGS-NFM purge/flush guidance. The raw 14-point set departs from the independence target: permutation Moran’s I (Anselin, 1995) yields $I_X = +0.40$ ($p = 0.003$), $I_Y = +0.50$ ($p = 0.001$), $R = 0.82$ — driven by the wall-stuck cluster (#10–13 within ~0.5 m, Fig. 2b). As a sensitivity analysis with this post-hoc cluster excluded, the same statistics drop to $I_X = +0.26$ ($p = 0.05$), $I_Y = +0.20$ ($p = 0.22$), $R = 0.90$, closer to the simulator’s design behaviour; the raw value remains the primary statistic. Six wall-hugging modes were logged in the post-trial debrief and are closed in the next firmware revision (stuck-motor diagnostic + escape-spin). Capital cost remained at ~5 % of the closest commercial alternative.

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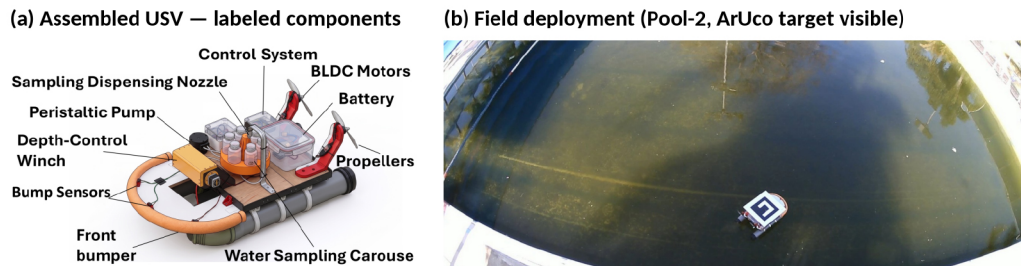


Figure 1. (a) Labelled exploded view of the assembled low-cost USV. Catamaran hull on two PVC pontoons; the surface platform carries the ESP32 control box, the 7-position water-sampling carousel with sample-dispensing nozzle, the stepper-winch depth probe (the depth-setting tube driven by a stepper motor on a winch drum), the front bumper with bump sensors used by the bump-and-turn rule, the battery pack, and twin DC motors with air propellers for thrust. (b) Field deployment of the same platform in the 12.5 × 20 m half-Olympic Pool-2 (28-04-2026); the white ArUco target on the deck is visible to the overhead camera used for the field trajectory in Fig. 2b.

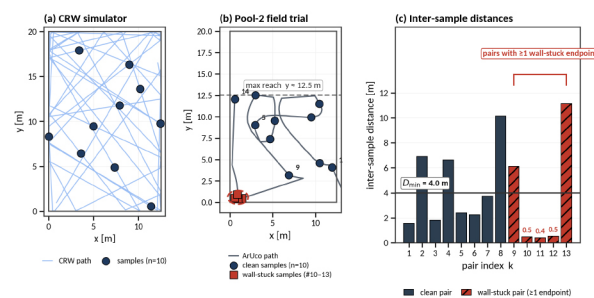


Figure 2. Design verification and field validation. CRW = correlated random walk; wall-stuck = sample logged while the platform was immobilised against the wall (samples #10–13 here); pair $k = \#k \rightarrow \#k+1$, shown red and hatched iff at least one endpoint is wall-stuck (so pair 9 = #9 → #10 is red because endpoint #10 is wall-stuck); design threshold $D_{\min} = 2\ell = 4.0$ m follows from the conservative correlation length $\ell = 2$ m used at design time. (a) CRW-simulator design path on the same 12.5×20 m pool (40 min, 10 stops; $\bar{L}_X = +0.04$, $\bar{L}_Y = +0.10$; $R = 1.59$). (b) Pool-2 field trial (28-04-2026, 12.5×20 m half-Olympic pool): 147 ArUco-tracked positions as one continuous trajectory with 14 sample stops; wall-stuck samples #10–13 are red squares inside a dashed red ellipse; samples #1, #5, #9 and #14 are labelled (trajectory endpoints and midpoints). The dashed grey line marks the boat's maximum northward reach in the 9-min window. (c) Inter-sample distances vs. $D_{\min}$. The red bracket spans pairs 9–13 (the segment touching the wall-stuck cluster, including the recovery jump on pair 13 = #13 → #14). Statistics are reported in the body. Clark–Evans $R = 0.82$ raw, 0.90 excluded.

Conclusion and Aquaculture Relevance

A USV that is simultaneously low-cost, QA/QC-compliant and statistically auditable lowers the practical barrier to dense, multi-depth water-quality monitoring in small and medium pond aquaculture. By replacing manual grab sampling with autonomous, ground-truth-verified bottle collection, the platform makes gradient-resolved measurements of dissolved oxygen, temperature, ammonia and emerging contaminants tractable for facilities that cannot justify stationary commercial samplers. In future trials, exclusion criteria will be pre-specified against the documented wall-hugging failure modes, eliminating any post-hoc selection.

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COMMUNICATING SCIENCE: THE IMPORTANCE OF MEETING JOURNALISTIC STANDARDS**

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For decades, trade magazines have served as a central communication platform for industry, delivering timely news, technical features, market insights and sector representation. Historically, these publications - predominantly in print - acted as the connective 'glue' within industries, informing stakeholders, shaping discourse and reflecting both technological and political developments.

The rapid rise of digital communication has fundamentally altered this landscape. Today, organisations, associations and individuals can publish and distribute content instantly to global audiences through websites, social media and professional platforms. This shift has challenged traditional publishing models, requiring established magazines such as *International Aquafeed* to adapt while maintaining the editorial standards and credibility associated with print.

This presentation examines the transition from print to digital publishing within the aquaculture sector and explores the continuing relevance of traditional media. It considers how audience engagement has evolved, particularly in response to search engines, SEO-driven content discovery and, more recently, AI-driven generative engines that deliver rapid, curated responses with embedded references.

Central to this discussion is the importance of applying journalistic standards when communicating scientific and technical information to non-specialist audiences. By bridging scientific communication and journalistic practice, this work highlights how clarity, accuracy and presentation can significantly improve the reach, impact and uptake of technical information in a rapidly evolving media environment.

Key Practical Considerations

1. Identifying the target audience

Effective communication begins with a clear understanding of the intended audience. Whether addressing editors, journalists, industry professionals, or consumers, each group has distinct editorial expectations. Tailoring content to these needs significantly improves the likelihood of publication, citation and wider dissemination.

2. Preparing content

Scientific findings must often be translated into accessible, engaging formats. This requires clear structure, concise language and logical flow. Short sentences, well-defined paragraphs and consistent style help present complex information in a form that is both readable and relevant to non-specialist audiences.

3. Presenting content

Consistency in formatting and presentation is essential. Submissions should follow a clear layout, including author identification, contact details and location. Opening paragraphs should be concise and engaging, while accuracy and clarity remain paramount. Supporting materials such as images should be properly captioned, numbered and supplied in high resolution to meet editorial standards.

4. Distributing content

Understanding distribution channels is critical. Social media platforms, digital publications and traditional print outlets each have distinct requirements. Direct communication with named editors, supported by a clear covering message and properly formatted attachments, increases the likelihood of successful publication. Visual content, including images and video, plays an increasingly important role in audience engagement.

5. Adapting scientific content for media use

There is significant value in repurposing scientific material for broader audiences. Abstracts can be adapted into introductory summaries, while traditional section headings such as 'Methods' and 'Results' may be replaced with more engaging, reader-focused subheadings. Emphasis should be placed on outcomes and practical implications rather than formal academic structure.

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6. Maximising reach and impact

Publication should not be viewed as a single event but as part of a broader communication strategy. Sharing content across multiple platforms, encouraging reposting and clearly referencing original publication sources enhances visibility while maintaining transparency and professional integrity.

Acknowledgement

The author acknowledges the many colleagues encountered and contributors to his experience over 45 years in journalism, whose commitment to professional standards continues to underscore the importance of a free and responsible press in a democratic society.

NEW FISH SPECIES ASSEMBLAGES FOR POND AQUACULTURE ADAPTED TO CLIMATE CHANGE CONDITIONS

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Context: extensive fish pond production in Europe and France

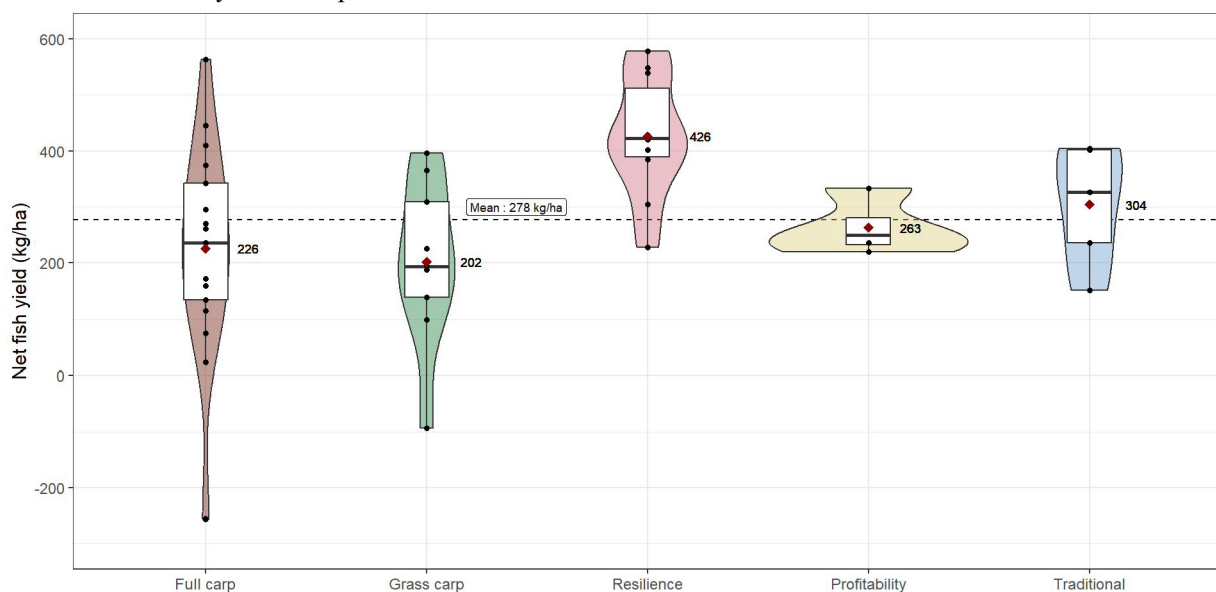
Historically, Europe has been home to many pond landscapes, usually used for extensive fish farming (Aubin *et al.*, 2017). This is the case for the Auvergne-Rhône-Alpes region of France, which contains several pond areas (Dombes, Forez, and Dauphiné). Fish farming persists in these areas but faces many challenges, including the impacts of climate change. In these extensive production systems, up to 70% of productivity comes from natural resources provided by the pond ecosystem (Robin *et al.*, 2016). This natural productivity can be enhanced through several management practices (liming, fertilization, feeding, and a dry period every 4-5 years) (Wezel *et al.*, 2013; Girard *et al.*, 2024). However, stocking strategies (fish species composition and density) are not always adapted to the productive potential and ecological functioning of individual ponds.

Materials and Methods

The FISHMIX project (2023–2026) aimed to evaluate new fish species assemblages suited to ponds, their productive potential, and their biological functioning, in the context of climate change. Over a three-year period, we monitored 46 fish ponds in the Dombes region, in which five scenarios of fish species assemblages were implemented. These assemblages were designed in collaboration with fish farmers, removing species that are more vulnerable to climate change, adding species uncommon in the region, and adjusting the proportions of the species. Fish production (yields and economic margins) and ecological parameters (water and sediment physicochemistry, phytoplankton, zooplankton, macrophytes, and macroinvertebrates) of all ponds were measured. We also monitored meteorological parameters and water temperature throughout the production season.

Figure 1:

Violin plots of net fish yields of 46 ponds stocked with one of five scenarios of fish species assemblages: full carp (80-90% common carp), grass carp (traditional scenario + grass carp), resilience (5-6 species, with two size cohorts for each), profitability (tench, whitefish, and largemouth bass), and traditional (60-70% common carp, 20-30% tench and whitefish, and 10% pike or pikeperch). Whiskers equal 1.5 times the interquartile distance. The dashed horizontal line shows the mean net fish yield of all ponds.



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Results and Discussion

Temperature and precipitation varied greatly among the three years, which caused pond functioning to vary greatly as well, thus illustrating impacts of climate change. Low water levels in ponds, especially at the end of winter, and high temperatures during the summer limited fish production. Despite high variability among sites and years, one highly diversified species-assemblage scenario (called “resilience”) showed promising performances, with a higher mean net fish yield (Figure 1) and economic margin.

The data generated by the project highlighted direct impacts of climate change on pond functioning and productivity potential. The increase in mean temperatures observed, along with prolonged droughts and heat waves, is forcing managers to rethink species assemblages. Introducing new species to the region such as largemouth bass (*Micropterus salmoides*), which is more resistant to high temperatures, or grass carp (*Ctenopharyngodon idella*), a herbivorous species that uses natural food resources not used by other fish species, may represent promising adaptation options. In light of these changes in production, market perspectives must also be adjusted. The scenarios tested provide potential pathways for sustainable pond aquaculture that combine acceptable farm income, production resilience, and ecosystem services such as biodiversity conservation.

Acknowledgments

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ECONOMIC ANALYSIS OF OPTICAL DELOUSING AS A CONTROL STRATEGY AGAINST SALMON LICE

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Introduction

Parasitic salmon lice (*Lepeophtheirus salmonis*) remain a significant economic and welfare challenge faced by the salmon farming industry. Studies show that salmon lice account for a significant proportion of biological losses and the consequent loss of revenue to the industry (e.g. Walde et al. 2023). While reactive treatments can mitigate these losses (Mustafa 2001) the most common types of treatments used today have been linked to an increased prevalence of injuries and wounds, reduced growth, and increased mortality rates (Overton et al. 2001). In response to this welfare paradox, several innovative technologies for the prevention or control of salmon lice infestations are increasingly utilized. While some of these have been shown to successfully reduce the infestation burden or need for reactive treatments (e.g., Cerbule & Godfroid 2020; Grøntvedt et al., 2018; Stien et al., 2016; Worm et al., 2026), studies investigating the economic impact of such strategies are still scarce. Yet, this knowledge can unlock the potential for production optimization, providing an invaluable contribution to the future of salmon farming. We therefore provide an economic analysis of one of these innovative technologies: optical delousing. Optical delousing is a non-handling, in-pen, control strategy in which laser pulses are used to remove lice from salmon and has previously been shown to decrease by half the number of reactive treatments (Worm et al. 2026).

Methods

We used a bioeconometric model incorporating baseline mortality and temperature-dependent growth for the grow-out phase, with temperature predictions for each of the 13 production areas (PAs) defined by the Norwegian Traffic Light System. We parametrized the model with empirical data from the literature and from reports submitted by farmers to the Norwegian government, and calculated production cost, revenue and net profitability for the production cycles obtained. To contrast the cost of salmon lice under scenarios in which production cycles utilize or not optical delousing as control strategy, we simulated 2000 reactive treatment schedules per scenario. We used temperature-dependent weekly probabilities of treatment obtained from Worm et al. (2026), where treatment probability depends on the use of the control strategy. Based on the literature, we assumed treatments incurred lost feeding days (i.e., lost growth), excess mortality, and decreased product quality at harvest. Frequencies for different treatment types were calculated from official reports (BarentsWatch, n.d.), and excess mortality rates per treatment type were obtained from Walde et al. (2021).

Results

Our simulations recovered the expected effect of the control strategy on reducing the number of treatments across all 13 production areas analysed. This reduction in treatments due to the use of the control strategy led to a ca. 45% average (across the 2000 simulated treatment schedules for each of the 13 PAs) mitigation in the excess mortality resulting from treatments observed in scenarios when no control strategy was deployed. This resulted in a mean increase in live biomass at the time of harvest of over 400 tones, and a mean increase in profit of 3.79 (NOK kg gutted⁻¹). This increase in profit was despite the increase in total costs resulting from the added cost for the control strategy, as well as the increase in cost for feed, due to the reduction in lost feeding days.

Conclusions

Our results demonstrate that optical delousing is an economically viable control strategy. The economic benefit of control incorporates increased growth by avoiding lost feeding days, reduction of handling-related mortality, and better product quality at harvest. Even more, our results reinforce the idea that investing in welfare of farmed fish can provide good economic returns to the industry.

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LCDV - INDUCED GENOTOXICITY IN GILTHEAD SEABREAM: EFFECTS OF DIETARY PHYTOBIOTICS

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Introduction

Lymphocystis disease (LCD), caused by the lymphocystis disease virus (LCDV), affects over 125 wild and cultured fish species and represents an important health and economic concern in Mediterranean seabream aquaculture (Borrego et al, 2017, Muniesa et al, 2010). LCDV–host interactions involve apoptosis and cellular DNA damage responses, which may contribute to infection-associated genotoxicity (Lilley et al, 2007, Hu et al, 2004). This study evaluated the effects of dietary *Origanum vulgare* and *Cinnamomum zeylanicum* essential oils on LCDV-induced genotoxicity in the liver and spleen of infected gilthead seabream.

Materials and Methods

The study was conducted in the experimental aquarium facilities (EL-43BIO/exp-01) of the Aquaculture Laboratory, Department of Ichthyology and Aquatic Environment, University of Thessaly, Greece, in accordance with the EU Directive 2010/63/EU. All procedures were performed by trained personnel under the supervision of FELASA-accredited scientists. Gilthead seabream (*Sparus aurata*) (M.W.: 5±0.32g) experimentally infected with LCDV were fed diets supplemented with *Origanum vulgare* or *Cinnamomum zeylanicum* essential oils at 1% and 2%, administered either preventively (90 days before infection) or therapeutically (at infection). The 11 experimental groups included naïve, infected, recovered, and control fish (Figure 1). Infection prevalence was assessed every 15 days post-infection, and cumulative mortality was calculated at the end of the experiment. Following the experimental period (90 days post-infection (dpi)), liver and spleen samples were collected from three fish per aquarium for DNA damage assessment using the comet assay. DNA damage was quantified as the percentage of tail DNA using fluorescence microscopy and Comet Assay IV™ software. Data were analyzed using one-way ANOVA followed by Tukey's post hoc test.

Results

LCDV clinical signs appeared from 15 dpi, with a significantly lower prevalence in prophylactically treated fish, particularly CIN90.1 ($p < 0.05$). Mortality was highest in the OR1, OR2, and PC groups, whereas prophylactic treatment, especially CIN90.1 and CIN90.2, markedly reduced mortality to control levels. The infected groups exhibited significantly higher DNA migration than the non-infected NC and RF groups in both liver and spleen cells ($p < 0.05$). The experimental diets did not induce higher genotoxicity compared to the positive control group in either organ. However, in the CIN90.1 group in spleen cells, the DNA damage was clearly pronounced ($p < 0.05$). The comet assay indicated increased DNA fragmentation in spleen cells compared to than in hepatocytes. Additionally, no differences were observed in DNA strand breakage between the non-infected NC and RF groups. Genotoxicity induced in both liver and spleen cells following experimental infection of gilthead seabream fed with diets supplemented with essential oils is shown in Figure 1.

Discussion

LCDV infection increased DNA damage in both liver and spleen cells, with a stronger response observed in the spleen. This may reflect the spleen's major role in antiviral immunity, haematopoiesis, and viral replication, whereas the liver primarily performs metabolic and detoxification functions (Valverde et al, 2017, Zhang et al, 2023). Therapeutic supplementation with *O. vulgare* and *C. zeylanicum* reduced DNA damage compared to that in the positive control, suggesting a protective effect against LCDV-associated genotoxic stress. Notably, CIN90.1 showed high DNA damage but very low mortality, potentially indicating an enhanced host response that limits disease severity. Overall, the findings suggest tissue-specific genotoxic responses to LCDV and a modulatory effect of phytochemicals on infection-related DNA damage.

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NEUROTOXICITY AND BEHAVIOURAL EFFECTS OF BIO-BASED MICROPLASTICS IN FISH: JUVENILE GILTHEAD SEABREAM (*Sparus aurata*) AS CASE STUDY

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Introduction

The problematic of plastic litter in aquatic environments is well-established and studied. Conventional petroleum-based plastics are associated with a high carbon footprint, environmental persistence and microplastic pollution. These microplastics can affect behavioural and neurological functions, metabolism, and intestinal permeability of fish (Jacob et al., 2020). Farmed fish species are of major concern, since they can be exposed to microplastics directly through seawater, from fragmentation of aquaculture infrastructures, daily maintenance materials, and aquafeeds. Additionally, microplastic presence in such species poses further risks for human health, due to the consumption of contaminated products (Mossotto et al., 2025).

In recent years, there has been an increasing concern to develop bio-based, sustainable and environmentally-friendly alternatives to conventional petroleum-based plastics. However, as any other disposable material, these bio-alternatives might accidentally end up discarded into marine environments and fragment into microplastics. The current knowledge is very limited in regard to the toxicological effects of bio-microplastics in fish species, especially concerning behavioural and neuronal alterations. Accordingly, it is essential to validate the eco-friendly claims of these emerging bio-based plastic alternatives.

The present study investigated the neurotoxicity and behavioural effects of chronic oral exposure to different bio-microplastics in the commercially and ecologically-relevant fish species gilthead seabream (*Sparus aurata*).

Materials and Methods

Farmed *S. aurata* juveniles (18.78 ± 0.51 cm total length, 103.46 ± 5.52 g weight) were kept in recirculating aquaculture systems (RAS) under optimal rearing conditions. During the 28-day exposure trial, specimens were fed 1% body weight with a commercial-based diet (CTR) or with three contaminated diets, containing either i) polylactic acid microplastics (PLA); ii) polypropylene 2,5-furandicarboxylate microplastics (PPF), or iii) mixed polymer PLA/PPF microplastics. The three contaminated feeds contained ~60 particles g⁻¹ feed, each ranging between 500 and 1000 µm.

During the trial, swimming activity was assessed twice a week (n = 8 fish per treatment) through direct observations to evaluate behavioural alterations elicited by bio-microplastics. After 28 days of trial, fish (n = 7 per treatment) were randomly collected and euthanized for dissection and collection of plasma and brain tissue. To evaluate the neurotoxic and stress effects of biobased microplastics, the following endpoints were assessed: brain condition index; plasmatic cortisol levels; neurotransmission (acetylcholinesterase activity, AChE); antioxidant defences (superoxide dismutase activity, SOD; catalase activity, CAT; glutathione S-transferase activity, GST; and glutathione peroxidase activity, GPX); and cell damage (ubiquitin content, UBI; lipid peroxidation, LPO; and caspase activity, CASP). Statistical differences between treatments were analysed through Generalized Linear Mixed Models (GLMMs) for swimming activity data, or via one-way ANOVA for all other endpoints.

Results and Discussion

No statistical differences were found for swimming activity, brain-somatic index, AChE activity and cortisol concentration. These findings indicate that the bio-microplastics investigated do not severely alter neural transmission, affect the overall physiological condition of the brain, or elicit a generalized stress response in seabream. Concerning antioxidant defences, all three biopolymers increased the activity of CAT, GST and SOD. This activation of the antioxidant systems is most likely related to an increase in reactive oxygen species (ROS) in brain cells. However, severe lipid damage did not occur (i.e. no increase in LPO).

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Although data analysis is still ongoing, preliminary results suggest that the exposure to PLA, PPF and the mixed polymer do not lead to severe toxic effects in brain health and function in *S. aurata*. These findings are optimistic for the bioplastic industry, which should strive to develop and produce plastics that are not only more environmentally sustainable, but also pose no harm to wildlife. Equally, these findings are useful to the aquaculture industry, since it should shift towards the use of bioplastics in tools and packaging that are non-toxic to farmed species. Nonetheless, these results need to be complemented with further research on other *S. aurata* tissues and other aquaculture-linked species.

Acknowledgment

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TRANSGENERATIONAL EFFECTS OF OCEAN WARMING ON MATERNAL PROVISIONING IN SEA URCHINS

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Introduction

Seawater temperature is a critical environmental factor that significantly affects the fitness and aquaculture industry of commercially important marine invertebrates, including sea urchins. Early life stages are particularly vulnerable to ocean warming (OW), which can reduce survival and growth by up to 80% under 2-4°C increases. Increasing broodstock food resources and availability may reduce the negative impacts of OW by increasing larval tolerance. Emerging studies assess offspring success after pre-exposing parents to warming conditions, which suggests the existence of beneficial carryover effects known as transgenerational effects. However, whether transgenerational acclimation to OW and nutrition is possible in sea urchins remains largely unknown (Zhao et al., 2018). Because sea urchins cannot synthesize carotenoids *de novo*, dietary provision of these pigments is essential as they play a critical role in egg production and offspring quality (Shpigel et al., 2006). This study evaluated whether maternal conditioning to high and low carotenoid diets influences the resilience of *Paracentrotus lividus* (Lamarck, 1816) larvae to increased temperatures, aiming to optimize hatchery production strategies against OW.

Materials and Methods

Paracentrotus lividus broodstock (N=120) was acclimated in recirculating aquaculture systems (RAS) and reared for four months on two temperature scenarios: local environmental temperature (18°C) and predicted warming temperature 21.5°C (+3.5°C), following IPCC predictions for 2100. During conditioning, the individuals were fed with two isoproteic and isolipidic formulated diets: a high carotenoid diet (HC) enriched with *Nannochloropsis* sp., and a low carotenoid diet (LC). At the end of the conditioning trial, proximate composition of female gonads ((e.g., carotenoids; eicosapentaenoic acid - EPA; total polyunsaturated fatty acids-PUFA; omega-3 (n-3) PUFA)) was assessed. Sea urchins were induced to spawn through injection with 0.5 M KCl in the peristomal membrane. To isolate maternal investment and minimize paternal genotypic variance, all eggs were fertilized using a sperm pool from males conditioned at 18 °C x HC. Following spawning, oocytes (n = 50 eggs per female) and fecundity were quantified in four females per broodstock treatment. The larval experiments followed a 2x2x2 factorial approach. Larvae from each maternal conditioning treatment were exposed to both 18 °C and +3.5 °C. Within these temperature treatments, two distinct larval nutritional treatments were established to assess larval survival: 1) an exogenous nutrition treatment, where larvae were reared under a standard mixed microalgal diet (*Chaetoceros calcitrans* and *Rhodomonas lens*), and 2) an endogenous nutrition treatment, where larvae were left unfed to isolate and quantify the direct impact of maternal egg reserves. Larval rearing was conducted in 600 mL beakers stocked at 2.5 larvae/mL. To evaluate survival, larvae from each treatment were sampled at the 4, 6, 8-arm and competence stages, with the corresponding age in days post-fertilization (DPF) recorded.

Results

Conditioning temperature and diet significantly influenced the proximate composition of female gonads. Females fed HC diet and exposed to +3.5°C showed enhanced accumulation of carotenoids (67.46 µg.g⁻¹ DW), EPA (10.68%), n-3 PUFA (16.45 %), and total PUFA (36.60 %). Egg diameter (90-97 µm) was not affected by temperature or diet treatments. Fecundity was affected by diet, with the highest yields observed in females fed the HC diet and exposed to 18 °C (8.4 × 10⁴ eggs.g⁻¹). Maternal conditioning had a significant influence on larval survival across both fed and unfed treatments. When females were conditioned at 18 °C, fed larvae from both HC and LC diets reached the competence stage at 20 DPF

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when reared at 18 °C. However, survival was higher in larvae from the HC diet, with 13 %. Conversely, when exposed to +3.5°C, larvae from the LC diet stopped development at the 4-arm stage, while those from the HC diet developed to the 6-arm stage and reached 16 DPF with a survival of 9%. A similar pattern was observed when females were conditioned at +3.5 °C. When larvae were reared at 18 °C, fed larvae from both maternal diets achieved competence at 20 DPF, with higher survival observed on those from the HC diet (13%). However, when kept at +3.5°C, larvae from LC diet stopped at the 4-arm stage and died by 8 DPF, while larvae from HC reached the 6-arm stage and reached 20 DPF with 3.39% survival. Among the unfed larval treatments, maternal HC diet consistently extended larval longevity. When females were conditioned at 18 °C and fed an HC diet, their unfed larvae survived until 12 DPF in both temperature conditions (18 °C and +3.5 °C). Similarly, when females were conditioned at +3.5°C and HC diet, unfed larvae survived until 12 DPF at 18°C and until 10 DPF at +3.5°C.

Discussion

This study demonstrates that the synergistic effects of temperature and diet quality strongly influence maternal provisioning and offspring resilience. Specifically, a combination of an HC diet and + 3.5°C was found to significantly enhance the accumulation of carotenoids and critical fatty acids (EPA, n-3 PUFA, and PUFA) in female gonads. This represents a maternal investment strategy to help offspring cope with thermal stress. This was further evidenced by larval survival rates under different temperature conditions. Fed larvae from females conditioned to the HC diet and +3.5°C achieved the competence stage with significantly higher survival rates when reared at 18°C compared to larvae from the LC diet. These maternal carry-over effects were evident when the larvae were exposed to +3.5°C, with higher survival capacities observed in both fed and unfed larvae. These findings highlight that optimizing broodstock nutrition is a vital strategy to trigger beneficial carry-over effects that enhance larval survival. Incorporating high-quality diets into hatchery protocols offers the aquaculture industry a promising solution to support production and build resilience against increasingly challenging environmental conditions.

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AQUASCAPE—MAPPING THE WORLD'S AQUACULTURE.

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Introduction

The bulk of aquaculture production takes place in developing nations (FAO, 2024), whereas most of the tools used to support the activity have been developed in Western countries (Ferreira et al., 2012). The consequence is an information divide that presents a major challenge for sustainable growth of the sector.

The use of mathematical models to simulate growth and environmental effects of cultivation, both for licensing and monitoring, is far more prevalent—and consigned in legislation—in countries such as Norway and Canada than in Asia and South America. The use of such tools extends to nations such as the US and various EU Member-States where aquaculture production is insignificant both from a global perspective and to their internal economies.

These issues should be a key part of the sector's agenda for sustainability over the next decades (Costa-Pierce et al, 2026).

There is widespread use of informal licensing approaches for aquaculture in many parts of the world, both on land and in coastal waterbodies—this is a problem on many levels, including assessment of accurate production data and waste footprints, biosecurity, and access to private capital. Tools based on remote sensing, machine learning, and AI can be leveraged on a global scale to provide solutions to some of these challenges. This paper describes the development of AquaScape, a global mapping tool designed to address a number of important issues in this domain, and exemplifies how it has been applied in collaboration with the Aquaculture Stewardship Council (ASC) to improve the quality of certification of shrimp culture in India.

Methods

AquaScape (<https://longline.co.uk/aquascape/>) was originally conceived as a platform for communicating science to management, using a mapping metaphor to provide a simplified representation of complex simulations. This involved sophisticated scientific and technical requirements whereby the outputs of hydrological, hydrodynamic, and ecological models (Nobre et al., 2010) needed to be archived, retrieved, and synthesised on the platform—the technical challenges included accurate geospatial storage of aquaculture structures such as ponds and cages, overlaying model results using cutting-edge design, and since the platform is web-based, optimising retrieval and display speed.

Since Indonesia was the first country where AquaScape was applied, this was fundamental in bridging the information gap referred above; the various simulation tools and their outputs were displayed in the context of the world's second largest aquaculture producer (14.9 X 10⁶ t including seaweeds, FAO, 2024), which allowed a comprehensive understanding of scaling and complexity and presented an enormous computational challenge to ensure a rich and responsive user experience.

The lack of geodata on aquaculture structures of different types was identified as a key gap for management, and it was recognised that little could be achieved in the application of more sophisticated models without a solid geographical baseline.

The resulting registry was stored in the AquaScape platform, which now contains about 3.5 million aquaculture structures throughout the world, corresponding to thirty million tonnes of annual production, or 32 % of world production of aquatic animals (94.4 X 10⁶ t, FAO, 2024).

Ponds, cages, shellfish rafts, and other structures (**Error! Reference source not found.**) were mapped using a combination of remote sensing, human validation, and machine learning. Currently, AI plays a major role in improving data quality and helping to identify key features in the dataset that can be used to convert data into information.

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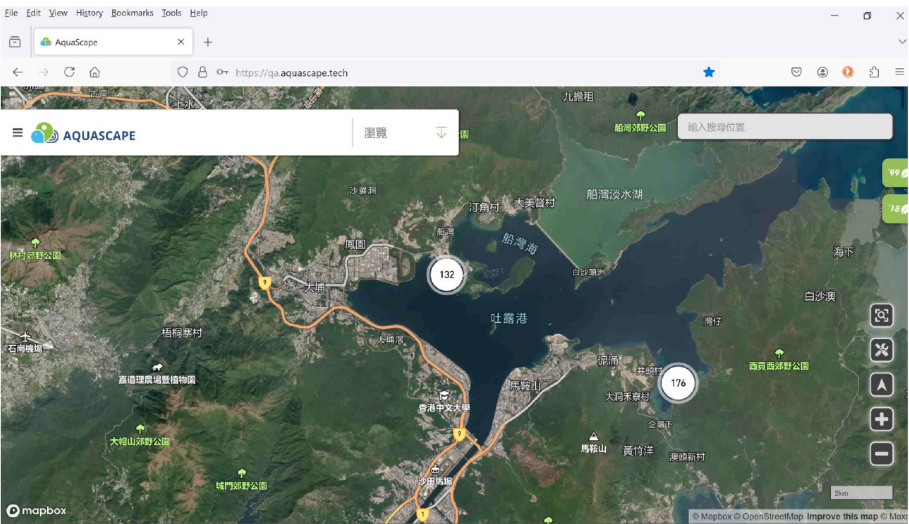


Fig. 1. Fish farm clusters in an area of the South China Sea under Hong Kong jurisdiction.



Fig. 2. AquaScape registry of shrimp ponds in Andhra Pradesh, India, showing data tags for a selected production unit.

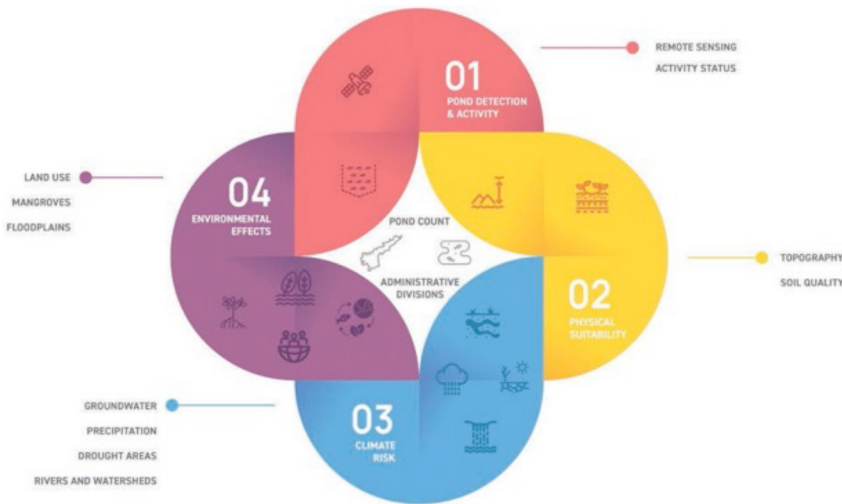


Fig. 3. Schematic representation of landscape connectivity analysis.

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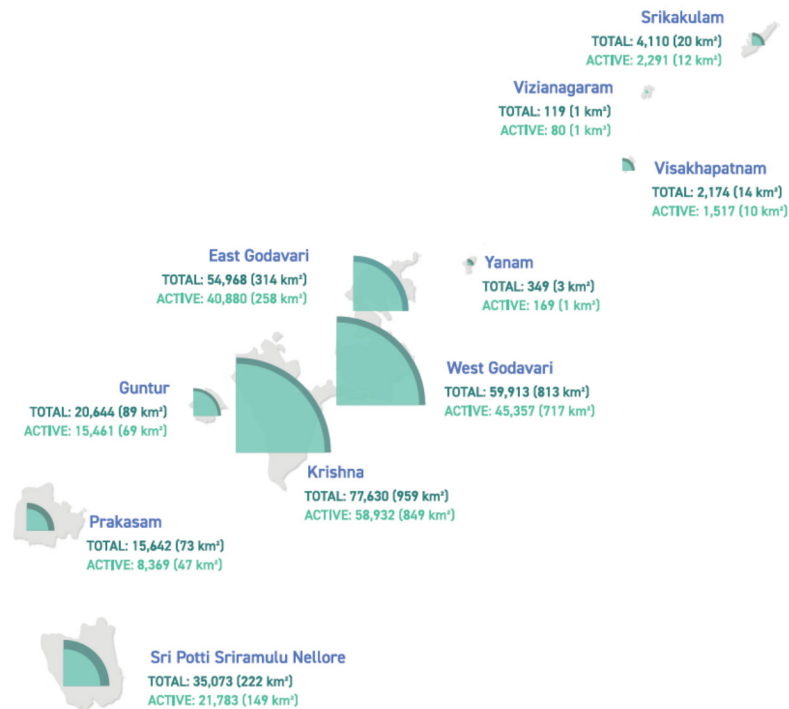


Fig. 4. AquaScape assessment of pond numbers and activity status by district in Andhra Pradesh.

AquaScape is now realising its potential as a platform for sustainability; it (i) enables informal aquaculture to be part of a registry, using a tag-based system to provide standard information such as area and volume, production status (e.g. for shrimp ponds: intensive, semi-intensive, fallow), and where applicable, lease data; and (ii) allows for models to be applied at scale to determine production, dissolved and particulate emissions, connectivity, and other key performance indicators.

An example of this kind of potential application was the connectivity assessment in Andhra Pradesh (India) executed by the ASC and Longline.

This application used data from AquaScape for shrimp pond location, complementary remote sensing data and machine learning to examine activity status, using e.g. aeration and water and vegetation colour indices, and combining these outputs with GIS multicriteria evaluation to determine suitability.

Synthesis of case-study outcomes

270,622 ponds were identified in the coastal districts of Andhra Pradesh using pond detection analysis, with over 97% used for shrimp production. Total activity in each district averaged 72% in 2022 (Fig. 1).

All these ponds are connected to their surrounding landscape through land, water, habitat, and climate. Farming on unsuitable land or within a 100-metre buffer zone from sensitive features (as per ASC standards) raises ecological and environmental concerns, along with highlighting significant risk to the sector due to climate change.

Srikakulam, Sri Potti Sriramulu Nellore, and Yanam are the most promising districts in Andhra Pradesh for potential certification and further shrimp farming investment due to their favourable land conditions, limited environmental conflicts, and potential to revitalise unused ponds. SPS Nellore stands out for its ideal soil, flat terrain, and low ecological impact, while Srikakulam and Yanam offer suitable sites with manageable climate risks and minimal mangrove loss.

East Godavari also has strong potential, though it requires careful planning to address environmental risk in some areas. In contrast, districts like Krishna, Vizianagaram, and West Godavari face significant challenges from mangrove degradation, flood risk, and regulatory complexity, making further expansion more difficult without major environmental restoration.

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Remote sensing has emerged as a powerful tool for monitoring and analysing aquaculture activities and their broader environmental and socio-economic contexts. By leveraging satellite imagery and geospatial analysis, this approach provides scalable, cost-effective, and data-driven insights into aquaculture farming operations and their interactions with the surrounding landscape.

This Environment Intelligence approach can be broadly applied at scale and used to evaluate very large production areas.

Environmental Intelligence can thus be used to bridge the information divide between sustainability tools and major farming areas to improve the quality of local management and global certification.

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ALGAE-ENRICHED DIETS AS A NUTRITIONAL STRATEGY TO MODULATE WOUND HEALING RESPONSES IN ATLANTIC SALMON

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INTRODUCTION

Atlantic salmon (*Salmo salar*) aquaculture faces significant challenges associated with skin integrity loss, driven primarily by sea lice (*Lepeophtheirus salmonis*) infestation, but also mechanical handling and crowding stress. Skin lesions constitute major welfare and biosecurity concerns, functioning as entry points for opportunistic pathogens and are directly linked to reduced growth, increased mortality, and substantial economic losses. The wound microenvironment involves dynamic shifts in local microbial community composition alongside a transcriptional immune response both at the lesion site and in surrounding epithelial tissue, processes that have been increasingly characterized in recent years. Functional feeds represent a promising non-pharmacological strategy to actively support skin recovery following injury. Marine algae are of particular interest given their diverse bioactive compounds — including sulfated polysaccharides, phlorotannins, fucoxanthin, EPA, and β -glucans — with documented immunomodulatory, anti-inflammatory, and antimicrobial properties. *Ascophyllum nodosum*, *Phaeodactylum tricornutum*, and *Chaetoceros muelleri* have shown relevant biological activity in *ex vivo* assays and preliminary feeding trials, yet their therapeutic capacity to modulate wound healing at both the microbial community and transcriptional level in Atlantic salmon needs to be further explored. The present study evaluates the effect of dietary supplementation with these three algae, on the wound healing response in Atlantic salmon subjected to wound model, characterizing wound microbiota and local transcriptional profiles to provide an integrated assessment of host–microbiome dynamics during cutaneous recovery.

METHODS

Atlantic salmon were subjected to a two-step wound protocol, as an attempt to mimic the establishment of a sea lice-associated lesion, combining a standardized perifocal abrasion of mucus and scales followed by a central punch biopsy. Immediately following wounding, fish were assigned to one of four experimental diets administered throughout the 45-day recovery period: a control diet (CTRL) and three diets supplemented with *Ascophyllum nodosum* whole biomass (ASCOP), *Phaeodactylum tricornutum* hydrolysed biomass (PHAEO), or *Chaetoceros muelleri* broken biomass (CHAETO). Wound closure was monitored photographically over the full recovery period, encompassing the main healing stages: re-epithelization, inflammation, granulation, and maturation. At key time points corresponding to each stage, wound tissue samples were collected for microbiota community profiling and transcriptional analysis of stage-specific marker genes.

RESULTS

Photographic analysis of wound closure indicates a trend towards faster healing in fish fed PHAEO and CHAETO diets compared to CTRL and ASCOP. Transcriptional profiling across the four healing stages revealed differential expression of genes associated with immune activation (*il1b*, *tnfa*, *mhcII*), inflammatory regulation (*il10*, *il6*, *cox2*, *ptgs2*), and tissue remodeling (*mmp9*, *mmp13*, *fn1*, *krt*) between dietary groups. Microbiota community composition at the wound site differed among treatments, with further characterization currently underway. These findings suggest that therapeutic dietary inclusion of selected microalgae, particularly *P. tricornutum* and *Chaetoceros muelleri*, initiated immediately following injury, may actively support wound healing in Atlantic salmon. These results point to the potential of algae-based functional feeds as a post-injury nutritional intervention with practical welfare implications for salmon aquaculture. Further analysis will clarify the microbial and transcriptional mechanisms underlying the observed differences in healing dynamics.

Acknowledgment

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AN *EX VIVO* SKIN EXPLANT MODEL TO EVALUATE WOUND RE-EPITHELIALISATION AND INFLAMMATORY PRIMING IN ATLANTIC SALMON

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INTRODUCTION

Skin wound healing in Atlantic salmon (*Salmo salar*) is a multistep biological process encompassing re-epithelialisation, inflammation, granulation tissue formation and tissue remodelling. Impairment of any of these phases' compromises barrier restoration and fish welfare, with significant consequences for performance and resilience against pathogens like the ectoparasite sea lice. Comparative studies between salmonid species provide compelling insights into the biology of resistance. Coho salmon display near-complete resistance to sea lice, associated with a rapid epithelial hyperplasia response driven by accelerated keratocyte migration during re-epithelialisation (Salisbury et al., 2024). Furthermore, coho salmon mount a rapid, well-localised, neutrophil-dominated inflammatory response within 24–48 hours of louse attachment, whereas Atlantic salmon exhibit a delayed and poorly localised reaction that facilitates parasite establishment (Sveen et al., 2025). These observations collectively suggest that both the kinetics of re-epithelialisation and the quality of early inflammatory priming are critical determinants of effective skin defence. We hypothesise that targeted dietary interventions can recapitulate, in Atlantic salmon, key aspects of the wound-healing phenotype observed in resistant salmonid species. To test this, we employ an Atlantic salmon skin explant model, as New Approach Methodologies (NAMs), enabling mechanistic, tissue-level interrogation of nutritionally driven repair processes while reducing reliance on *in vivo* experimentation. Two complementary explant-based trials specifically address the identified rate-limiting steps in skin defence: (i) acceleration of keratocyte migration during re-epithelialisation (Trial 1), and (ii) induction of a rapid, acute inflammatory response at the wound site (Trial 2).

METHODS

Skin sections from Atlantic salmon were collected in the area between the dorsal fin and the lateral line and used as a standardised liquid-submerged skin explant culture system using an inert, non-porous support to maximise tissue viability and functional performance. Skin dissection procedures, ex-vivo wound infliction and culture conditions had a specific emphasis on preserving the skin secretome (i.e. the full set of proteins and metabolites released by the explant). Bacterial growth in culture media was monitored by optical density at 600 nm and maintained below 15% relative to baseline through scheduled medium changes. Explant viability (72 hours) was assessed via metabolic activity (Alamar Blue - resazurin assay) and membrane integrity (total protein release). Tissue stress and goblet cell activity were evaluated by quantifying total secreted mucus in the culture medium using Alcian Blue or Periodic Acid–Schiff staining coupled to colorimetric analysis. Healthy explants were defined by sustained metabolic activity and limited protein leakage throughout the assay duration. Following SOP establishment for maximal basal viability, the skin explant functional performance was used to gain new insights on the role of bioactive compounds with demonstrated or hypothesised capacity to enhance keratocyte migration (Trial 1) and compounds signalling a pathogenic insult (PAMPs), and a positive immunological control. Endpoints across both trials included gene expression profiling using panels targeting skin re-epithelialisation and inflammatory pathways.

RESULTS

Differential effects of the tested compound categories on the transcriptional upregulation of genes associated with keratocyte motility, cytoskeletal remodelling and epithelial proliferation generated new insights, on the mechanisms to accelerate the re-epithelialisation process in a wounded skin. Moreover, PAMP-based dietary treatments and insult signalling compounds primed a faster and localised neutrophilic response at the wound site, reflected in earlier upregulation of acute-phase inflammatory genes. Together, these new generated data provide integrated evidence in Atlantic salmon that several compounds (with the potential to be afterwards delivered via feed) have the potential to accelerate the re-epithelialisation phase and amplify early inflammatory priming following standardised skin wounding.

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CONCLUSION

This work establishes Atlantic salmon skin explants as a practical NAM-based platform to investigate nutritionally driven modulation of skin wound healing. The data indicate that selected bioactive compounds can accelerate re-epithelialisation and enhance early inflammatory priming following skin injury, two processes that are critical for effective barrier restoration and characteristic of resistant salmonid species. These findings support the use of explant models as a screening and mechanistic tool to prioritise functional ingredients for subsequent feed formulation and *in vivo* validation. Ultimately, this approach may accelerate the development of nutritional strategies aimed at improving skin integrity, welfare, and resilience to ectoparasites in commercial salmon aquaculture.

Acknowledgment

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DOPAMINE INHIBITION INCREASES THE SEEKING FOR BODY TACTILE STIMULATION IN AN IMPORTANT COMERCIAL MARINE SPECIES, THE GILT HEAD SEABREAM SPARUS AURATA

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Introduction

Tactile stimulation (TS) is defined as a positive sensory experience resulting from physical contact (touch) between individuals or via artificial devices (Bolognesi et al., 2019; Soares et al., 2011). TS is involved in various relationships between animals, such as grooming in primates (Dunbar, 2010), cooperative interactions between fish (Bshary and Würth, 2001), therapeutic massage in humans (Field et al., 2005), and in the context of animal husbandry (Gauy et al., 2022). In these contexts, TS plays a crucial role in promoting animal welfare and strengthening social bonds. To the moment, the association between DA and TS has only been observed in cleaner-client context, i.e. always the presence of a fish as a “reward” (Messias et al., 2016a, 2016b). Here, we aimed to test if the TS per se can have the rewarding role, not given by other living organisms but provided by an artificial apparatus. Thus, we investigated the role of DA in modulating the response to tactile stimuli and the choice of TS in an important commercial species of teleost fish, the gilthead seabream (*Sparus aurata*). We predicted that if TS is processed as a reward (or positive valence) and modulated by exogenous changes in available DA levels, blocking endogenous levels would function as an omission of reward, resulting in behavioural compensation characterised by the seeking the physical stimulation. Conversely, increased DA levels would induce the opposite effect.

Materials and methods

Fish were first exposed to an artificial apparatus consisting of a rectangular PVC frame fixed in the centre of the aquarium, with vertically attached sticks lined with silicone bristles providing TS as fish swam through them. Groups of four individuals received seven days of TS and were then randomly assigned to one of three exogenous treatments: D1 agonist, D1 antagonist, or saline (12 groups per treatment). Fish then returned to an aquarium containing an apparatus with sticks half with/half without silicone bristles for analysis of post-treatment preference (60 min of video recording).

Secondly, to test the efficiency of our DA exogenous manipulations, we needed to understand the effects that intramuscular injections would have on the DA brain level of *Sparus aurata* individuals, which areas would be affected, and how long the effects would last. Four timepoints were defined at which the fish would be euthanised by deepening anaesthesia, following an intramuscular injection of one of three treatments: 15 minutes, 30 minutes, 45 minutes and 60 minutes. Six individuals were euthanised at each time point for each compound, totalling n = 24 in each treatment, for subsequent brain HPLC analysis of DA and DOPAC.

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Results and discussion

We found that all treatments sought the TS at a similar frequency but those under DA blockage showed a greater relative preference for the TS area, having a higher relative frequency of crossing the bristles than those receiving other treatments. In the second experiment, fish treated with D1 blocker showed a significant reduction in DA concentration in the forebrain after 60 min, confirming the antagonist's effectiveness. These results suggest that TS is associated with dopaminergic reward pathways in fish, similarly to mammals. Although TS in the natural environment of *S. aurata* is not described in the literature as in other species (see Soares et al., 2011), it seems however able to generate positive responses associated with reward. It is thus possible that TS occurs in a more subtle way for *S. aurata*, such as through touch between conspecifics and elements present in their natural environment, or even from water movements that produce tactile perception between individuals. Our results suggest that body tactile stimulation enrichment may have a great potential for aquaculture application, particularly in this important commercial species.

Acknowledgments

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DIFFERENT EFFECTS OF BODY TACTILE STIMULATION ON THE WELFARE OF THE ORNAMENTAL ANGELFISH AND THE SIAMESE FIGHTING FISH

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Introduction

Aquaculture of ornamental fish is among the fastest-growing activities worldwide. Nevertheless, the several stages of the ornamental fish trade can impair fish welfare, from initial capture to final housing. In this context, environmental enrichment is a key approach to mitigate such negative impacts, particularly in housing aquaria. Recently, body tactile stimulation (TS), akin to gentle touch or therapeutic massage, has emerged as a promising tool of sensory enrichment for fish. Studies have shown that artificial TS reduces stress in reef species (Soares et al., 2011), diminishes fear in zebrafish (Schirmer et al., 2013), and limits aggression while promoting growth in Nile tilapia (Gauy et al., 2022; 2023). Considering that enrichment can affect species differently, we evaluated the effects of TS on aggressive behavior and stress in two widely reared ornamental species, the Siamese fighting fish (*Betta splendens*) and the cichlid angelfish (*Pterophyllum scalare*). Given that monoamines mediate TS mechanisms in vertebrates, we also investigated the association between TS and brain Dopamine (DA) and serotonin (5-HT) levels, specifically in *B. splendens*.

Methods

Fish were exposed for 22 days to either an artificial TS apparatus or to an identical one without bristles, serving as a control (Fig. 1). Then, we evaluated individual aggressive behavior using mirror tests and pair fights, and stress by cortisol levels in both species. Brain DA, 5-HT, and their metabolites were measured by HPLC only in *B. splendens* due to logistical reasons.

Results and discussion

TS reduced aggression in male angelfish without altering cortisol levels, whereas in *B. splendens*, it increased restrained fighting and cortisol levels. Brain monoamines analysis in *B. splendens* revealed decreased 5-HT and increased 5HIAA/5HT turnover. DA levels remained unchanged, indicating no activation of the neural reward pathway. These contrasting outcomes may reflect species-specific differences in ecological history and behavioral traits. *P. scalare*, for instance, inhabits macrophyte-rich environments where substrate contact is frequent. Conversely, artificial selection for heightened aggressiveness or prolonged social isolation may render TS stressful rather than rewarding for *B. splendens*. Overall, our findings suggest that the efficiency of tactile enrichment is species-dependent, highlighting the importance of tailoring welfare interventions accordingly.

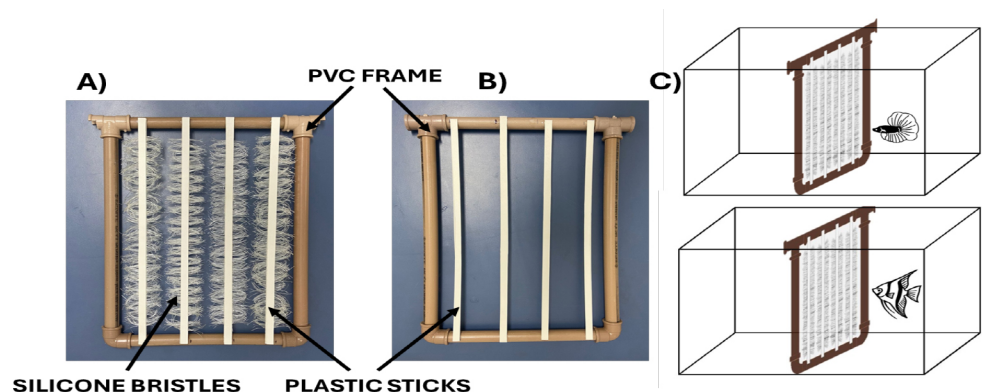


Figure 1 – Tactile stimulation. The TS apparatus consisted of a rectangular PVC frame filled with vertical plastic sticks, with silicone bristles on their sides that promote TS (A), and without silicone bristles for control (B). Both apparatuses were placed in the middle of the tank, and fish received TS when passing through the bristles (C). N = 20 *per treatment, per species*.

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FREE CHOICE OF BODY TACTILE STIMULATION ENHANCES WELFARE OF NILE TILAPIA

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Introduction

Body tactile stimulation (TS), similar to gentle touch or therapeutic massage, has emerged as a promising form of sensory enrichment for fish. Previous studies have shown that artificial TS alleviates stress in reef fish (Soares et al., 2011), reduces fear-related behavior in zebrafish (Schirmer et al., 2013), and mitigates confinement stress in gilthead seabream (Soares et al., 2026). In Nile tilapia, *Oreochromis niloticus*, TS has been shown to reduce aggressive interactions and enhance growth performance. However, these behavioral and productive benefits are not accompanied by a reduction in cortisol levels (Gaury et al., 2022, 2023). In the present study, we investigated whether allowing Nile tilapia to freely choose to receive TS would result in greater welfare benefits by reducing group aggression and cortisol levels. A preference test was used as it provides an important approach for assessing the affective valence of environmental enrichment, helping to determine whether animals actively seek or avoid a given stimulus and, consequently, whether it is likely to improve Nile tilapia welfare.

Methods

Groups of 4 adult male fish were exposed for 21 days to one of three treatments: “with TS”, “no TS”, and “choice TS” (Fig.1). A rectangular apparatus featuring plastic rods with silicone bristles on their sides (Fig.1A); without silicone bristles for control (Fig. 1B); and a half-with / half-without silicone bristles to allow fish choice (Fig. 1C). The apparatuses were placed in the middle of the tank (Fig. 1D), and fish received TS when passing through the bristles. Aggressive interactions were video recorded (10 min each) every three days. Biometric measurements and blood samples were collected at the beginning and end of the period to analyze growth and cortisol levels, respectively.

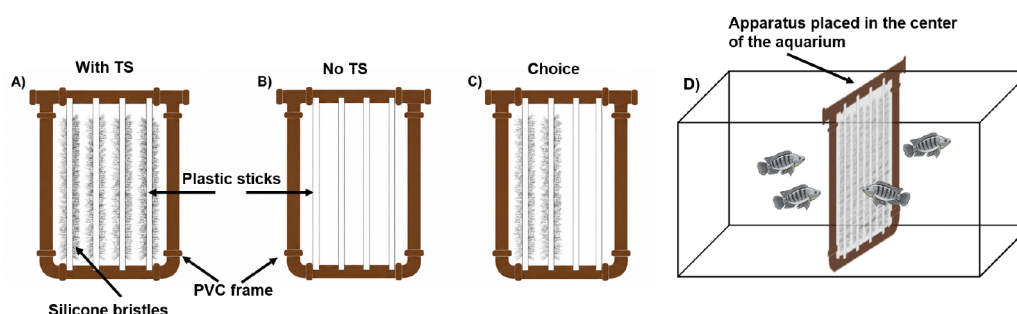


Figure 1 – Tactile stimulation. The TS apparatus consisted of a rectangular PVC frame filled with vertical plastic sticks, with silicone bristles on their sides that promote TS (A), without silicone bristles for control (B), and a half-with / half-without silicone bristles to allow fish choice. The apparatus were placed in the middle of the tank, and fish received TS when passing through the bristles (an example in D). “no TS” (n = 13), “with TS” (n = 15), and “choice TS” (n = 14) groups *per* treatment.

(Continued on next page)

Results and discussion

Fish crossed by the apparatus more frequently in the “no TS” than in the “with TS” treatment. In the choice treatment, fish passed through the bristles side beginning with ~10% and increasing to ~40% over time. One-way ANOVA revealed that group aggression was higher ($p < 0.0001$) and the specific growth rate was lower in the “no TS” treatment compared to the other two treatments ($p < 0.01$). These variables were similar between the “with TS” and “choice TS” ($p > 0.21$). We also found a positive Spearman rank correlation between crossings by the apparatus and aggressiveness in the “no TS” treatment; no significant correlations in “with TS” treatment, whereas a negative correlation was found in the “choice TS” treatment for bristles choices. Repeated measures ANOVA showed that cortisol levels increased in the “no TS” ($p = 0.003$) and “with TS” ($p = 0.03$) groups. Nevertheless, cortisol remained unchanged in the choice group ($p = 0.38$) and was lower than the other two treatments ($p < 0.01$). We conclude that allowing Nile tilapia free choice of tactile stimulation (TS) yields more beneficial outcomes by reducing aggressiveness and social stress while enhancing growth. Overall, free-choice TS represents a more effective environmental enrichment strategy for this species.

Acknowledgment

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PHENOLIC PLANT EXTRACTS AND PHARMACOLOGICAL ACHIEVEMENTS IN THE BACTERIAL AND VIRAL DISEASES IN THE *Litopenaeus vannamei* JUVENILE PRODUCTION IN MEXICO

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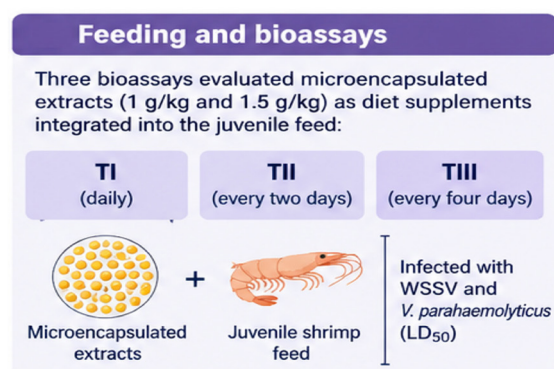
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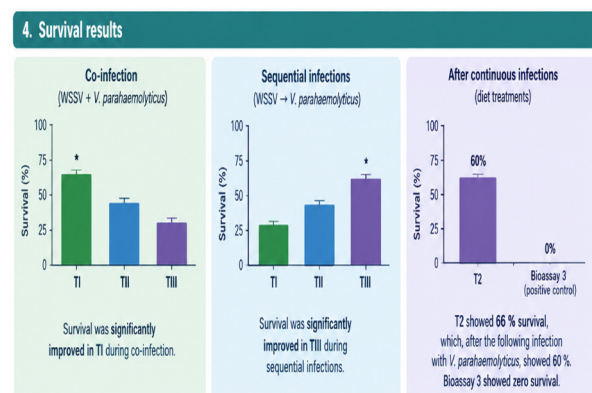
Introduction

Shrimp juvenile rearing is one of the most important processes of shrimp aquaculture worldwide, enhancing the production of millions of tons of shrimp annually and providing employment for thousands of people (Cuéllar-Lugo et al., 2018). Throughout history, multiple diseases caused by various viruses and bacteria have led to millions of dollars in crop losses. To combat viruses and bacterial diseases in juvenile production instead antibiotics that have increased pathogen resistance (Morales-Covarrubias et al., 2011) the use of phenolic extracts plant extracts in Mexico due to their antibacterial and antiviral pharmacological properties (Banerjee et al., 2008) has shown successfully results. In the present study, the effects of the addition of microencapsulated phenolic extracts from plant species from the tropical deciduous forest in Mexico on the growth, survival, and gene expression of shrimp juvenile has been determined.

Material and Methods



Results



Discussion

The higher concentrations of total phenols, flavonoids, and condensed tannins in *M. emarginata* leaves than those reported by Vasavilbazo-Saucedo et al. (2018) could be related to the ecological characteristics of the sampling sites. Those authors collected samples on a sandy island without a high diversity of herbivores and with extreme dry weather. In contrast, the specimens collected in this study face higher herbivore competition, less water stress, and parasites. These factors stimulate phenolic compound production as a defense mechanism (Nieto Ramírez et al., 2018).

(Continued on next page)

No previous reports regarding *P. blakeana* have been published. Compared with other species from the Pereskioideae subfamily, such as *Opuntia* spp, *Cylindropuntia rosea*, *Tacinga inamoena*, and *Austrocylindropuntia cylindrica*, the phenolic compound profiles vary (Chen et al., 2023; Dantas et al., 2013; Ko et al., 2025; Pensamiento-Niño et al., 2021; Torres et al., 2023; Vieira et al., 2024). These phenolic compounds variation has been shown to affect antioxidant capacity and antiviral or antibacterial activities. In this sense, Zhang et al. (2016) and Sanathkumar et al. (2014) have reported significantly higher mortality compared to the present results in shrimp co-infected with WSSV and *V. parahaemolyticus* applied to *P. vannamei* post-larvae. This difference could be attributed to the fact that simultaneous infections with two major pathogens tend to trigger an antagonistic interaction, reducing the effect or response of one of them in the shrimp (Rubio-Castro et al., 2016). Consistent with this, Pang et al. (2019) who supplemented feed with phosphatases, corroborated this phenomenon and a lower mortality in organisms infected simultaneously by WSSV and *V. parahaemolyticus* compared to those subjected to sequential infections at different times and in different order were reported.

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SEAWEEDS AND DUNE COASTAL PLANTS: ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL

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Seaweeds and dune coastal plants have seen an increase in interest within the medicinal field, due to the identification of a variety of bioactive compounds (Narayanan et al., 2025; Holdt & Kraan, 2011). However, common extraction methodologies require the use of organic solvents that can be harmful to human health and the environment. Extracts performed from extraction techniques following the principles of green chemistry (Chemat et al, 2012) remain understudied. In this study, 5 species of seaweeds and 6 species of plants found along the Portuguese coastal dunes, were selected. Extracts were carried out using the green extraction techniques subcritical water extraction (SWE) and ultrasound-assisted extraction (UAE) under multiple extraction conditions. For UAE the influence of extraction time and solvent to solid ratio was evaluated. The influence of temperature and pressure was evaluated for SWE. Phenolic content and antioxidant activity of the extracts were determined by TPC method and by DPPH[•], ABTS^{•+}, [•]NO and [•]OH scavenging assays. Chemical composition of the plant extracts was determined by HPLC-DAD and LC-MS. The antimicrobial potential of the seaweed extracts was tested on *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Salmonella enteritidis* and *Escherichia coli*. The best conditions of extraction were determined based on the antimicrobial and antioxidant potential of the extracts. Following the conclusion of this research, the extracts will be evaluated for antimicrobial activity against the fish pathogen *Vibrio sp.* and potential inclusion in feeds for *Gilthead seabream* will be investigated.

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This work received financial support from project “NUTRIBRAIN - Coastal Flora for Mental Well-being Promotion: Development of food supplements based on dune plant compounds with impact on BDNF-TrkB signalling” (COMPETE2030-FEDER-00686600, operation n° 15759), supported by FEDER (COMPETE 2030) and national funds by FCT / MECI. The authors also thanks the support of PT national funds (FCT/MECI) through the project UID/50006/2025 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos. K. G. thanks FCT/MCTES for the financial support through a PhD fellowship (2024.16226.PRT). C. G. is thankful for her contract (2020.03436.CEECIND/CP1596/CT0008, DOI 10.54499/2020.03436.CEECIND/CP1596/CT0008) financed by FCT/MCTES—CEEC Individual 2020 Program Contract.

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SEAWEEDS AND DUNE COASTAL PLANTS: ANTIOXIDANT AND ANTIMICROBIAL POTENTIAL

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Seaweeds and dune coastal plants have seen an increase in interest within the medicinal field, due to the identification of a variety of bioactive compounds (Narayanan et al., 2025; Holdt & Kraan, 2011). However, common extraction methodologies require the use of organic solvents that can be harmful to human health and the environment. Extracts performed from extraction techniques following the principles of green chemistry (Chemat et al, 2012) remain understudied. In this study, 5 species of seaweeds and 6 species of plants found along the Portuguese coastal dunes, were selected. Extracts were carried out using the green extraction techniques subcritical water extraction (SWE) and ultrasound-assisted extraction (UAE) under multiple extraction conditions. For UAE the influence of extraction time and solvent to solid ratio was evaluated. The influence of temperature and pressure was evaluated for SWE. Phenolic content and antioxidant activity of the extracts were determined by TPC method and by DPPH[•], ABTS^{•+}, [•]NO and [•]OH scavenging assays. Chemical composition of the plant extracts was determined by HPLC-DAD and LC-MS. The antimicrobial potential of the seaweed extracts was tested on *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Salmonella enteritidis* and *Escherichia coli*. The best conditions of extraction were determined based on the antimicrobial and antioxidant potential of the extracts. Following the conclusion of this research, the extracts will be evaluated for antimicrobial activity against the fish pathogen *Vibrio sp.* and potential inclusion in feeds for *Gilthead seabream* will be investigated.

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INTEGRATING DUCKWEED INTO INTENSIVE CATFISH CULTURE THROUGH CLOSED-LOOP WATER RECYCLING

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Introduction

Intensive production of African catfish, *Clarias gariepinus*, generates water containing suspended solids and dissolved nutrients, particularly nitrogen and phosphorus compounds. Although recirculating aquaculture systems reduce water consumption, nutrient removal and the management of accumulated organic matter remain important operational and environmental challenges. Duckweed, particularly species of the genus *Lemna*, grows rapidly on nutrient-rich water and can assimilate dissolved nutrients into protein-rich plant biomass.

This work presents an integrated production concept combining indoor catfish culture in a recirculating aquaculture system, outdoor catfish tanks and duckweed cultivation within a single water-recycling loop. The objectives are to improve water reuse, recover nutrients and produce a locally available feed supplement for juvenile catfish.

System Concept and Operation

Water discharged from the indoor and outdoor catfish production units first passes through mechanical filtration for the removal of floating and larger suspended solids. The mechanically treated water, which retains dissolved nitrogen, phosphorus and other metabolites, is subsequently directed to shallow duckweed cultivation ponds.

Duckweed assimilates dissolved nutrients during rapid vegetative growth and thereby acts as a biological polishing stage. The treated water is then returned to both catfish production units, closing the water-recycling loop and reducing the requirement for fresh water and nutrient discharge.

Under favourable temperature, nutrient and light conditions, expected duckweed productivity is approximately 10–30 g dry matter m⁻² day⁻¹, with harvesting carried out every one to two days. Regular harvesting prevents excessive surface density and continuously removes assimilated nutrients from the water.

Duckweed Processing and Nutritional Use

Harvested duckweed is washed, dried and milled before being incorporated into formulated feed for catfish larvae and fingerlings. Depending on species and cultivation conditions, duckweed may contain approximately 35–45% protein on a dry-matter basis. It also provides essential amino acids, vitamins, minerals and carotenoids.

The harvested biomass can therefore partially convert nutrients originating from fish production into a valuable feed ingredient. Further trials will determine suitable inclusion levels and evaluate effects on feed intake, growth, feed conversion, survival, fish health and water quality.

Discussion and Conclusion

The proposed system links intensive fish production, water treatment and feed-resource generation in a circular production process. Duckweed closes part of the nutrient loop by recovering dissolved nutrients that would otherwise be discharged or require additional treatment. Its integration may reduce water exchange, nutrient emissions and operating costs while providing a renewable supplementary feed material.

The concept is particularly relevant for intensive inland aquaculture facilities seeking to improve resource efficiency without substantially increasing the complexity of water treatment. Operational validation will focus on nutrient-removal efficiency, duckweed productivity, seasonal stability and the nutritional value of the harvested biomass. The integrated approach offers a practical pathway towards more circular, water-efficient and resilient catfish production.

FIRST DETECTION OF *Streptococcus iniae* ASSOCIATED WITH DISEASE IN FARMED EUROPEAN SEABASS *Dicentrarchus labrax* IN EAST GREECE

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Introduction

Streptococcus iniae is a well-recognized bacterial pathogen affecting a wide range of marine and freshwater fish species, causing significant economic losses in aquaculture worldwide (Austin & Austin, 2016). It has been widely reported in species such as tilapia and rainbow trout, where it causes septicemia and meningoencephalitis (Austin & Austin, 2016; Agnew & Barnes, 2007). In the Mediterranean aquaculture industry, European seabass (*Dicentrarchus labrax*) is one of the most commercially valuable species; however, reports of *S. iniae* infections in this host remain scarce. The emergence and spread of *S. iniae* have been associated with environmental stressors, including elevated water temperatures and high stocking densities (Mian et al., 2009). The present study describes the first confirmed occurrence of *S. iniae* infection in farmed European seabass in marine aquaculture facilities in East Greece, highlighting its clinical, microbiological, and epidemiological characteristics.

Materials and Methods

During the summer of 2025, increased mortality (8-10%) was observed in seabass populations from two marine cage farms located in South-East Aegean (Greece). Samples of moribund European seabass (average weight 90–500 g) were collected and subjected to clinical and laboratory examination. Gross pathological assessment was performed, followed by bacteriological analysis using standard culture techniques on blood agar. Isolated colonies were identified based on colony morphology, Gram staining, and biochemical characteristics, and further confirmed by molecular methods, including PCR targeting 16S rRNA followed by sequencing (Mata et al., 2004). The genome of the isolate was extracted and subsequently sequenced using both short- and long-read technologies followed by hybrid assembly. Antimicrobial susceptibility testing was performed using the disk diffusion method.

Results

Affected fish exhibited clinical signs consistent with streptococcosis, including anorexia, lethargy, erratic swimming behavior, ocular opacity and exophthalmia. External examination revealed redness of the head, anemia of the gills, petechial hemorrhages on the operculum and at the base of fins. Internally, ascites, splenomegaly, discoloration of the liver, enteritis with distended intestine hemorrhages, serous fluid within the lumen and congestion of visceral organs were observed. Pure bacterial cultures yielded Gram-positive, β -hemolytic, catalase-negative cocci arranged in chains. Biochemical profiling and genomic analysis confirmed the isolates as *Streptococcus iniae*. Antimicrobial susceptibility testing showed sensitivity to florfenicol and oxytetracycline. The outbreak coincided with elevated seawater temperatures (25–27°C), a known predisposing factor for streptococcal infections (Agnew & Barnes, 2007).

Discussion

This study represents the first documented case of *S. iniae* infection in farmed European seabass in Eastern Greece, expanding the known host range and geographical distribution of this pathogen. The clinical signs and pathological lesions observed are consistent with previous descriptions of streptococcosis in other fish species (Austin & Austin, 2016; Agnew & Barnes, 2007). The emergence of *S. iniae* in seabass raises concerns regarding its potential impact on Mediterranean aquaculture, particularly under conditions of elevated water temperatures, which may favor disease outbreaks. Early diagnosis through combined bacteriological and molecular methods proved essential for accurate identification. Genomic analyses provided insights regarding the virulence factors of the isolate and its phylogenetic position. Environmental conditions, particularly increased water temperature, likely played a critical role in the onset of the outbreak, as previously suggested (Mian et al., 2009). The observed antimicrobial resistance patterns emphasize the need for responsible antibiotic use (CLSI, 2020; Shoemaker et al., 2001). Preventive strategies, including vaccination, biosecurity measures, and improved farm management practices, should be considered to mitigate future outbreaks. Further epidemiological studies are required to assess the prevalence and impact of *S. iniae* in Mediterranean seabass aquaculture.

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LIFE CYCLE ASSESSMENT OF EUROPEAN SEABASS (*Dicentrarchus labrax*) FED TWO MARINE PEPTIDES-SUPPLEMENTED FEED FORMULATION STRATEGIES

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Introduction

Fish meal is widely used in aqua feeds because of its high quality nutritional profile. However, the catch of wild fish is linked to substantial environmental concerns, notably pressure on wild fish stocks and marine ecosystems. Limiting reliance on fish meal while preserving zootechnical performance has therefore become a key lever for enhancing the environmental sustainability of aquaculture. Feed formulation approaches typically include reducing fishmeal inclusion levels, lowering the quality of marine-derived ingredients, or replacing marine proteins with alternative sources such as plant-based or functional ingredients. Although these strategies aim to mitigate the environmental impacts associated with fishmeal use, they must be evaluated using standardized assessment tools such as Life Cycle Assessment (LCA), which quantifies environmental impacts across multiple categories (e.g., climate change, eutrophication). This study evaluated through LCA the environmental impacts of two formulation strategies, by partially replacing fish meal or using a lower quality fish meal in the diet with a supplementation of a marine peptide concentrate (Pepsea F) in European seabass (*Dicentrarchus labrax*).

Material and Methods

Data from Bardera *et al.* 2026 (presented in a separate abstract for EAS 2026) were used for LCA, including fish performance, feed and some farm data. Experimental diets included Pepsea F (Adisseo) and consisted of two formulation strategies: (i) partial replacement of high-quality fish meal (with 68% crude protein), reducing inclusion from 20% to 10%, and (ii) substitution of fish meal quality, from 68% to 60% of crude protein, at a constant 20% inclusion level. All experimental feeds were formulated to be isoproteic, isolipidic and isoenergetic. Performance parameters, including feed intake, specific growth rate (SGR), and feed conversion ratio (FCR), were measured.

LCA for Pepsea F at plant gate was conducted following ISO 14040/44, PEFCR and FAO LEAP guidelines, where applicable. LCA from cradle to farm gate (per kg of fish) was performed using feed ingredient list, growth performance and feed efficiency outputs, and Turkish seabass farm data. GFLI v2.2 database (2024) was used, and LCA followed FAO LEAP (2020) and PEFCR guidelines, where applicable. Feed ingredient origins reflected Turkish, European or Global import values when no other data was available. Five impact categories were assessed: climate change, acidification, eutrophication freshwater and marine, and water use. In addition, the Environmental Return on Investment (EROI) was calculated as the ratio of the carbon footprint avoided at the animal product level to the impact generated by additive production at the plant level.

Results

At the cradle to farm gate level, formulation strategy (i) with partial replacement of fish meal combined with Pepsea F supplementation, led to a marginal reduction in climate change impacts, from 5.92 to 5.85 kg CO₂eq/kg fish (-1%), compared to the basal diet. This strategy also resulted in reductions in marine eutrophication (-12%) and water use (-3%), while slightly increasing acidification (+1%) and freshwater eutrophication (+1%) per kg of fish.

In contrast, formulation strategy (ii), based on fish meal quality substitution at constant inclusion levels, yielded more pronounced LCA indicators improvements. Climate change decreased from 6.08 to 5.66 kg CO₂eq/kg fish (7%). In addition, there was an improvement in acidification (-1%), freshwater eutrophication (7%), and water use (3%) compared to the basal diet. However, marine eutrophication was slightly higher for the lowquality fish meal supplemented with Pepsea F (+4% per kg of fish) relative to basal diet.

The EROI calculated for the first strategy (i) resulted in 6, while it reached 38 from the second strategy (ii), indicating that for an investment of 1 kg CO₂eq due to feed additive production, its use resulted in these studies in savings of 6 or 38 kg CO₂eq on the value chain depending on the formulation strategy.

(Continued on next page)

Discussion & Conclusion

These results show that, additionally to performance improvements obtained in Bardera *et al.* 2026 (EAS 2026), Pepsea F associated with different formulation strategies can reduce different LCA environmental impacts in European seabass, with magnitude depending on the strategy applied. The second strategy, having more significant effects on climate change, appears especially relevant for companies with carbon reduction objectives, as CO₂ emissions represent one of the main indicators used to evaluate corporate sustainability performance.

In contrast, the first strategy relied on partial fish meal replacement with soy protein concentrate ingredient, which increased the climate change impact of the feed compared to the basal diet. Consequently, despite improved fish performance associated with Pepsea F supplementation, the final climate change reduction at the farm gate was limited and considered non-significant. This finding aligns with Kok *et al.* (2026), who emphasized that substituting marine ingredients with plantbased alternatives can modify the distribution of environmental pressures across ecosystems. In particular, the use of soy imported from South America is still associated with landuse change impacts, highlighting the importance of carefully evaluating tradeoffs between marine resource use and terrestrial environmental constraints when designing more sustainable aquafeeds.

Besides, the magnitude of the observed environmental benefits is influenced by methodological assumptions. In the second strategy (ii), identical fish meal proxies from the GFLI v2.2 database were applied to both high and lowquality fish meal, due to the lack of supplierspecific data and differentiated datasets. This conservative assumption likely underestimates the environmental gains, as lowerquality fish meal, potentially derived from byproducts, can reasonably be expected to have a lower environmental footprint under economic allocation. Consequently, the mitigation potential associated with Pepsea F marine peptide supplementation and fish meal quality decrease may be greater than reported here.

Taken together, these findings illustrate both the potential and the limitations of LCA for assessing fish meal reduction strategies. Further studies combining LCA with complementary indicators, such as Fish-In Fish-Out ratios (FIFO) and emerging biotic resource depletion or biodiversity metrics, would enable a more comprehensive evaluation of feed reformulation strategies and their implications for marine resource use and ecosystem integrity.

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PRELIMINARY GENOMIC AND PROTEOMIC INSIGHTS INTO VIRULENCE-ASSOCIATED FEATURES OF *Vibrio harveyi* STRAINS ISOLATED FROM EUROPEAN SEABASS

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Introduction

Vibriosis caused by *Vibrio harveyi* is an important bacterial disease affecting Mediterranean aquaculture, including European seabass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*). Genomic and proteomic characterization of *V. harveyi* may contribute to a better understanding of its pathogenicity and virulence mechanisms.

Materials and methods

Two *V. harveyi* isolates, 94/17 and A27, isolated from diseased and clinically healthy European seabass in Croatia, respectively, were subjected to whole-genome sequencing using Oxford Nanopore MinION and Illumina HiSeq platforms. The genomes were de novo assembled and analysed for the presence of virulence-associated genes using the Virulence Factor Database (VFDB). To complement the genomic analysis, proteomic profiling of bacterial cell fractions was performed using liquid chromatography–mass spectrometry (LC–MS).

Results

A total of 226 and 227 virulence-associated genes were identified in strains 94/17 and A27, respectively. The distribution of virulence genes was highly similar between the two strains, indicating a largely conserved virulence-associated gene repertoire. Comparative genomic analysis revealed a broad repertoire of putative virulence-associated genes involved in motility and flagellar assembly, quorum sensing, secretion systems, iron acquisition, membrane-associated functions, and toxin production. Proteomic analysis identified 389 and 292 protein entries in strains 94/17 and A27, respectively. Integration of genomic and proteomic data demonstrated concordance for several major virulence-associated systems. Both strains expressed abundant flagellar proteins, including FlaB and additional flagellins, quorum-sensing-associated proteins LuxS and a LuxP-like autoinducer-2-binding periplasmic protein, as well as outer membrane and transport-associated proteins including OmpA, OmpK, OmpW and TolC-family proteins. Notably, strain 94/17 showed a distinct virulence-associated proteomic profile, including detection of a zonular occludens toxin family protein, the type II secretion system component GspG and the quorum-sensing regulator LuxT. In contrast, strain A27 showed proteomic evidence of an active type VI secretion system through detection of an Hcp1-family effector and the contractile sheath component TssC.

Conclusion

These findings indicate that the genomic virulence potential of the two *V. harveyi* strains is partially reflected at the protein level while also revealing strain-specific differences in the detectable virulence-associated proteome. The combined genomic and proteomic approach highlights toxin- and secretion-associated features in strain 94/17 and type VI secretion in strain A27 as particularly interesting targets for further functional investigation.

SURVIVAL RATES FOR *Penaeus vannamei* AND *Penaeus monodon*: EVIDENCE FROM ASC STAKEHOLDER ENGAGEMENT ACROSS ASIA AND LATIN AMERICA

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Survival rate is one of the most critical performance indicators in commercial shrimp aquaculture. It was formally incorporated into the Aquaculture Stewardship Council (ASC) requirements, in 2022, through minimum grow-out survival thresholds within its Shrimp Standard, ranging from $\geq 25\%$ for unfed, non-permanently aerated systems to $\geq 45\%$ for fed systems without permanent aeration, and $\geq 65\%$ for fed, permanently aerated ponds. Despite these benchmarks, published datasets often fail to capture the full variability observed under commercial farming conditions.

This study presents survival rate data for *Penaeus vannamei* (whiteleg shrimp) and *Penaeus monodon* (giant tiger prawn) collected directly from industry stakeholders across key producing regions in Asia and Latin America, providing a commercially grounded perspective on species and system performance.

Across all regions surveyed, survival rates under normal commercial conditions broadly ranged from 65–75%, with well-managed farms consistently achieving 80–90%, and disease outbreaks or environmental stressors reducing survival to below 60%. Considerable variation was recorded both within and between regions, reflecting differences in farming system intensity, species, climate, and disease pressure.

In Asia, *P. vannamei* farms in India reported average survival of 60–65%, with top-performing farms reaching 85–90%. Seasonal variation was marked, with summer crops in semi-intensive systems achieving 70–85% compared to 65–75% in winter crops, where monsoon rainfall and low temperatures in northern states increased the risk of crop failure. *P. monodon* performance in India diverged sharply by system type, with extensive farms relying on wild seed averaging just 30% survival, against 65–75% in semi-intensive operations in Andhra Pradesh and Gujarat. Vietnam reported strong *P. vannamei* performance at around 75%, while *P. monodon* survival of 60–80% across extensive, semi-intensive, and intensive systems represented a notable deviation from the generally lower performance observed for this species elsewhere. In Indonesia, successful *P. vannamei* crops achieved 75–85% survival, although disease events reduced this range to 60–75%. Thailand reported average *P. vannamei* grow-out survival of around 70%, with wide variability, and *P. monodon* survival reaching 85% in successful crops but averaging 65–75% in practice.

In Latin America, *P. vannamei* survival in Ecuador ranged from 75–80%, while Brazil showed strong performance of over 70%—occasionally exceeding 80%—outside of colder months, when White Spot Syndrome Virus (WSSV) outbreaks drove survival below 50% during affected periods. Mexico performed below regional peers, with survival of 55–70% at higher stocking densities in Sonora and 45–50% at lower densities in Sinaloa extensive fed systems. Honduras and Guatemala reported survival of 60–70% and 60–80% per cycle respectively, with inter-cycle variability in Guatemala reaching 60–90%.

Disease pressure, particularly WSSV, and seasonal environmental fluctuation emerged as the primary drivers of survival loss across both species and all regions. System intensity was consistently associated with improved outcomes, most notably in *P. monodon* culture.

The variability captured in this stakeholder-derived dataset highlights the gap between experimental benchmarks and commercial reality, and underscores the value of industry-sourced evidence in establishing meaningful regional performance expectations for the global shrimp farming sector.

Commercial shrimp survival is far more variable and context-dependent than current standard benchmarks suggest, with biological and environmental drivers outweighing system classification.

CIRCULAR FEEDING STRATEGIES INTEGRATING SEA URCHINS AND SEA CUCUMBERS IN INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA)

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Introduction

Integrated multi-trophic aquaculture represents one of the most promising approaches to improve the sustainability of marine farming by coupling fed and extractive species within the same production system. In this framework, organic by-products generated by one species can be reused as a trophic resource by another, reducing waste outputs while producing additional biomass of commercial value (Ridler et al., 2007). Low-trophic marine invertebrates, such as sea urchins and sea cucumbers, are particularly suitable for this approach because of their complementary feeding ecology and high market value (Grosso et al., 2021). *Paracentrotus lividus* is a valuable echinoid mainly fed on macroalgal resources, whereas *Holothuria tubulosa* is a deposit-feeder able to ingest and assimilate organic biodeposits, potentially acting as an extractive species in benthic integrated systems. However, the development of echinoderm co-culture still requires sustainable diets able to support growth while maintaining the circularity of the system. In this context, circular aquafeed ingredients such as probiotics and single-cell proteins may represent promising tools to improve nutrient utilization and reduce dependence on conventional marine-derived protein sources (Eroldoğan et al 2023). Therefore, this study aimed to investigate, for the first time, the growth performance and survival of *P. lividus* and *H. tubulosa* in co-culture using sustainable dietary supplements. Three macroalgal-based diets were tested as the sole food source to sustain both species rearing: a control diet (macroalgae), a probiotic-enriched diet and a diet enriched with methanotrophic bacterial single-cell protein.

Materials and Methods

Sub-adults of *Paracentrotus lividus* (N = 90) were collected from the central Tyrrhenian Sea, while sub-adults of *Holothuria tubulosa* (N = 90) were obtained through artificial reproduction at the Laboratory of Experimental Ecology and Aquaculture, University of Rome Tor Vergata. After acclimation at 20 ± 0.5 °C and a two-week fasting period, specimens were allocated to recirculating aquaculture systems for a three-month feeding trial. Three macroalgal-based diets, composed of *Ulva* sp. and *Laminaria* sp. in equal proportions, 1:1 dry weight, were tested: a control diet containing only macroalgae (CTL); a probiotic-enriched diet supplemented with *Bacillus subtilis*, *Lactobacillus casei* and *Lactobacillus plantarum* (PRO); and a single-cell protein-enriched diet supplemented with the methanotroph *Methylococcus capsulatus* (META). Sea urchins were reared and fed in floating baskets, while sea cucumbers were placed on the sediment-enriched tank bottoms. Sea urchin feces passing through the basket mesh represented the only food source for sea cucumbers. Growth was assessed through somatic growth rate in both species, while the gonado-somatic index was calculated for sea urchins. Absorption efficiency was estimated from the organic matter content of food and feces.

Results

Survival was high throughout the experiment, with only two *P. lividus* specimens dying during the first days and no mortality recorded for *H. tubulosa*. All diets supported significant somatic and gonadal growth in sea urchins and positive somatic growth in sea cucumbers. However, the methanotrophic-enriched diet produced the highest overall performance. In *P. lividus*, META resulted in the highest somatic growth rate and gonado-somatic index, reaching 0.61%, and 13.76%, respectively. Sea cucumbers also showed the highest growth under META, followed by PRO and CTL, with final biomass increases of 50.17%, 41.90% and 33.80%. Absorption efficiency was high in both species, especially under META, where sea urchins absorbed 58% of dietary organic matter and sea cucumbers absorbed 60% of sea urchin biodeposits. Overall, the META diet reduced residual organic waste to 17%, compared with 30% in PRO and 36% in CTL.

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Discussion

This study demonstrates for the first time that sustainable dietary supplements, particularly methanotrophic bacterial single-cell protein, can support echinoderm integrated multi-trophic aquaculture. The high survival of both *Paracentrotus lividus* and *Holothuria tubulosa* confirmed the compatibility of these species in co-culture and the suitability of the tested diets. The single-cell protein diet was the most effective treatment, significantly improving sea urchin somatic growth, gonadal development and absorption efficiency, with performances comparable to those reported for fishmeal-enriched diets (Fernandez and Boudouresque 2000). It also enhanced sea cucumber growth, producing higher somatic growth rates than previous integrated aquaculture trials (Tolon et al., 2017; Grosso et al., 2021). These results suggest that methanotrophic bacterial meal can represent a sustainable alternative to fishmeal protein in low-trophic aquaculture. The probiotic-enriched diet had species-specific effects. It did not significantly improve sea urchin growth compared with the control, suggesting that the selected strains and dose were not effective for *P. lividus*. Conversely, it improved sea cucumber growth and absorption efficiency, supporting the key role of bacteria in deposit-feeder nutrition. Overall, both supplemented diets enhanced the circularity of the system, but the single-cell protein diet provided the best production and bioremediation outcomes. Under this treatment, only 17% of the ingested organic matter remained as final waste, highlighting the potential of this co-culture system for circular and regenerative echinoderm aquaculture.

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AGRICULTURAL BY-PRODUCTS AS FEED FOR SEA CUCUMBERS IN BIOFLOC-BASED INTEGRATED MULTI-TROPHIC AQUACULTURE

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Introduction

Integrated multi-trophic aquaculture can improve the sustainability of marine farming by converting dissolved and particulate wastes from fed species into new biomass through extractive and low-trophic organisms (Chopin et al., 2012). In this framework, sea cucumbers are highly promising candidates because of their deposit-feeding behaviour and ability to process organic matter accumulated in sediments (Zamora et al., 2018). Among Mediterranean species, *Holothuria tubulosa* is particularly relevant for aquaculture diversification and bioremediation-oriented systems (Grosso et al., 2021). However, its successful integration into circular aquaculture requires suitable feeds that mimic natural detrital resources while enhancing the nutritional role of associated microbial communities. Biofloc-based diets offer a valuable approach because they rely on heterotrophic microbial biomass growing on organic carbon sources and nitrogen inputs (Avnimelech, 2015). In integrated systems, nitrogen-rich aquaculture waste can therefore be reused to upgrade low-value agricultural by-products into nutritionally enriched feeds. The carbon-to-nitrogen ratio is a key factor in this process, as it regulates microbial growth, nutrient immobilization and the final quality of the biofloc available to deposit feeders. This study tested the growth performance of *H. tubulosa* fed biofloc-based diets produced from agricultural by-products and enriched with nitrogen derived from aquaculture waste. Three plant-based diets were compared with a seagrass-detritus control to assess whether carbon-to-nitrogen optimization and microbial conditioning can support sustainable sea cucumber production within integrated multi-trophic aquaculture systems.

Materials and Methods

Specimens of *Holothuria tubulosa* were collected by scuba diving from infralittoral habitats from the central Tyrrhenian Sea and transported to the Laboratory of Experimental Ecology and Aquaculture, University of Rome Tor Vergata in aerated seawater under controlled temperature. After acclimation in quarantine tanks, specimens were fasted for 15 days to allow gut evacuation, then weighed and selected for the feeding trial. The experiment was carried out in independent recirculating aquaculture tanks adapted for sea cucumber rearing, each containing a fine silica sand substrate suitable for deposit-feeding activity and bacterial colonization. Four biofloc-based diets were tested: seagrass detritus as natural control, corn stalks with nitrogen addition, wheat husks with nitrogen addition, and corn stalks without nitrogen addition. Agricultural by-products were dried, ground, sieved and matured in aerated bioreactors with probiotic supplementation. Nitrogen, derived from aquaculture waste, was added to selected diets to optimize the carbon-to-nitrogen ratio and promote heterotrophic microbial development. Sea cucumbers were stocked at 500 g m⁻² and fed twice weekly. Water quality was monitored regularly, while survival and somatic growth were assessed monthly through drained weight measurements.

Results

The second feeding trial showed that all upcycled plant-based biofloc diets were suitable for *Holothuria tubulosa*, with 100% survival and positive somatic growth under all conditions. However, growth differed significantly among diets. Sea cucumbers fed *Posidonia oceanica* detritus showed the highest performance, with mean growth rates above 35%. Wheat husks with nitrogen addition supported similar growth, around 27%, and did not differ significantly from the natural control. Corn stalks with nitrogen addition also promoted high growth, around 25%, while corn stalks without nitrogen addition showed the lowest growth, around 19%, confirming the importance of nitrogen enrichment and carbon-to-nitrogen optimization.

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Discussion

The second trial showed that *Holothuria tubulosa* can efficiently exploit sustainable plant-based diets derived from agricultural by-products, confirming its trophic plasticity and suitability for low-trophic aquaculture. All diets supported positive somatic growth, but clear differences emerged according to substrate type and nitrogen availability. The highest growth was obtained with *Posidonia oceanica* detritus, confirming the ecological relevance of seagrass debris as a natural food source for Mediterranean sea cucumbers and providing a suitable reference diet (Boncagni et al., 2019). Notably, wheat husks and corn stalks enriched with nitrogen produced growth performances close to the seagrass control, despite their lignocellulosic and intrinsically low-protein composition. This suggests that microbial conditioning in biofloc systems can upgrade low-value agricultural residues into nutritionally effective feeds. The poorer performance of corn stalks without nitrogen addition confirmed that nitrogen availability and carbon-to-nitrogen optimization are key factors for biofloc development. A balanced carbon-to-nitrogen ratio likely stimulated heterotrophic microbial growth, improving substrate digestibility and generating protein-rich microbial biomass available to deposit feeders. Overall, these findings indicate that nitrogen-enriched agricultural by-products can support sustainable *H. tubulosa* aquaculture, while future metagenomic and transcriptomic analyses may clarify the microbial mechanisms driving nutrient upgrading.

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SPINAL HEALTH OF EARLY-LIFE PIKEPERCH IN RELATION TO GRADING STEPS: A PROJECT REPORT

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Introduction and Background

Aquaculture of pikeperch (*Sander lucioperca*) has advanced greatly during the last decades. However, some barriers remain, e.g. the production of sufficient larvae and strong fluctuations in their quality between the reproduction cycles. One issue is the variable and occasionally high occurrence of spinal deformities, which negatively influence animal welfare and production efficiency (Policar et al., 2019). In a pretrial study (Franz et al., 2025), the overlap of grading procedures with fundamental spinal development was found. Grading, although necessary to reduce cannibalism in this species, poses a risk to negatively influence the early developing structures of the spine. Consequently, this EPFC co-funded project follows up on the previous pretrial. It covers a series of grading steps of early-life pikeperch from a RAS hatchery, analysing their development, occurring spinal changes, and potential links to related factors as age, size group, and selected fatty acid (FA) influences (El Kertaoui et al., 2021). Here, project results are presented and potential adaptations in rearing procedures are discussed. This could help to reduce spinal deformities resulting from mechanical stress on these developmental stages of pikeperch, and simultaneously aid facility output and skeletal health of the animals.

Sampling and Methods

Specimen sampling encompassed a series of grading steps starting from the first (34 dph) to fourth (57 dph) routine size sortings within the facility. These consisted of three manual gradings, using grid boxes (first to third step), and machine grading (fourth step). Specimen were sampled from the resulting size groups, including normal and deformed spinal conditions. The analysis consisted of morphometric measurements, histological preparation of cleared and stained specimens to allow for spine morphology observations, and FA analyses of specimen from the groups and additional influential maternal and feed factors using gas chromatography (HR-GC).

Results and Discussion

Morphometric measurements highlighted the increasing size differences between the groups. Increasingly, differences in length between the deformed and non-deformed specimen within the same size groups were found. Body width comparisons indicated some level of squeezing being applied to the specimen during the grading steps. HR-GC data showed a pattern shift at around 42-43 dph, during the late larval to early juvenile transition. Hereby, the before trends in the developmentally influential polyunsaturated FAs and monounsaturated FAs proportions changed. We concluded this to indicate some transition either in the digestive or metabolism capabilities in these age groups (Reis et al., 2020). Skeletal analyses highlighted the regional specific occurrence of compressed and fused vertebrae, with compressions predominantly occurring in mid and posterior spine areas, while the less frequent fusions mainly occurred in the posteriormost sections. Analyses of single steps indicated that an accumulation of deformities occurs over time, highlighting the meaning of long-term observations. Within the earliest stages, another spinal variation type was observed in smaller specimen, showing their higher fragility.

Studies on skeletal deformations remains difficult, as influential factors are broad (Boglione et al. 2013). Here, no direct uniform influence of grading on spine deformities was found, however, indications for this further accumulated. Follow-up studies should take into account that control groups are practically unavailable, and are recommended to use the separation of spine areas in % of length regions. Still, the project could demonstrate that adaptations of grading are already possible within the given setup. These include postponing the first grading, and adapting the grid passages to more selectively remove largest specimen only. It was found that focusing on specimen with double the mean group length can be considered a conservative detection threshold for cannibals within the size groups.

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Acknowledgments and Project Output

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ADVANCES IN FISH CELL CULTURE SYSTEMS FOR SUSTAINABLE AQUACULTURE AND RESEARCH APPLICATIONS

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Introduction

The rapid expansion of global aquaculture has increased the need for innovative, sustainable and ethically responsible research tools. Fish cell culture systems have evolved into a versatile platform that bridges the gap between basic biology, disease research, toxicology and the production of alternative proteins. This study provides an overview of recent advances in the establishment, optimisation and application of cell lines derived from fish, with a focus on species of importance to European aquaculture.

We are investigating current methods for isolating primary cells, techniques for immortalisation, and the development of three-dimensional (3D) culture systems that better replicate the conditions found in living organisms. We place particular emphasis on the role of fish cell cultures in researching the interactions between pathogens and hosts, in vaccine development, and in environmental impact assessments, including responses to pollutants and climate-related stressors.

Furthermore, we discuss the potential of fish cell cultures in the emerging field of cellular aquaculture, highlighting challenges relating to scalability, cost-effectiveness and regulatory acceptance. Comparative analyses show that, whilst significant progress has been made in optimising growth media and bioreactor conditions, further research is required to develop commercially viable production systems. Overall, fish cell culture technologies represent a promising tool to enhance sustainability and innovation in aquaculture, and reduce animal experiments.

Results

At our laboratory at the Research Institute for Farm Animal Biology, Fish Growth Physiology group, a total of 63 fish cell lines from 17 fish species are currently stored. Of these, 55 cell lines derived from 13 species were established in-house, propagated, and cryopreserved as long-term cultures in liquid nitrogen ^[1-8].

All cell lines were characterised regarding to their growth behaviour and morphology. In addition, molecular validation using standardised genetic markers was performed to confirm their origin (DNA barcoding). Selected cell lines were further analysed in detail, including assessments of growth kinetics, mitochondrial function (e.g., using Seahorse extracellular flux assays), as well as protein and gene expression profiling.

Discussion

Fish cell culture systems represent a powerful tool to advance the principles of the 3Rs (Replacement, Reduction, Refinement) in aquatic research and aquaculture. First, they provide clear opportunities for replacement of live animal experiments in areas such as toxicology, pathogen screening, and environmental monitoring. In vitro models allow controlled, reproducible testing conditions and enable mechanistic studies that would be difficult to achieve in whole organisms.

Second, fish cell lines also play a key role in reducing animal experiments by minimising the number of animals needed for experimental validation and, in some cases, enabling full replacement. Once established, these cell lines can be propagated indefinitely, supporting high-throughput screening approaches. This capability reduces the need for repeated animal sampling across different experiments and laboratories.

Third, cell culture approaches support refinement by decreasing the severity of procedures involving live fish. Early-stage screening of stressors, pollutants, or pathogens can be conducted in vitro, thereby limiting in vivo experiments to only the most relevant conditions and reducing animal distress.

Despite these advantages, limitations remain. Fish cell cultures cannot fully replicate the complexity of whole organisms, including systemic immune responses, endocrine regulation, and tissue–tissue interactions. Therefore, they should be viewed as complementary tools within integrated experimental frameworks.

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Overall, the establishment of diverse fish cell lines—such as those presented in this study—substantially strengthens the toolbox for ethically responsible research. Continued development of advanced models (e.g., 3D cultures, co-culture systems) will further enhance their relevance and accelerate the transition toward more sustainable and animal-friendly aquaculture research.

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EFFECTS OF DIETARY RAW, YEAST-FERMENTED AND BROMELAIN-PROCESSED *Ulva* ON GROWTH, DIGESTIVE ENZYME ACTIVITIES AND INNATE IMMUNITY IN JUVENILE MEAGRE, *Argyrosomus regius*

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Introduction

Meagre (*Argyrosomus regius*) is an emerging Mediterranean aquaculture species characterised by rapid growth, favourable feed conversion and desirable flesh quality; however, species-specific functional feeds are needed to support its sustainable production (Güroy et al., 2024). Macroalgae, particularly *Ulva* sp., are promising functional ingredients because they provide nutrients, minerals, vitamins and bioactive compounds capable of supporting fish growth, immunity and antioxidant capacity (Wan et al., 2019). However, their nutritional effectiveness may be constrained by variable composition and nutrient accessibility, while biological or enzymatic processing may improve digestibility and functional value (Wan et al., 2019; Wiszniewski et al., 2019; Qudratullah et al., 2025). Therefore, this study compared raw, yeast-fermented, and bromelain-processed *Ulva* sp. products at three dietary inclusion levels in juvenile meagre.

Materials and Methods

Juvenile meagre with an initial body weight of approximately 5 g were randomly distributed into 400-L tanks at 15 fish per tank, with three replicates per treatment. In addition to a control diet, nine experimental diets were formulated with raw, yeast-fermented or bromelain-processed *Ulva* sp. products at inclusion levels of 2, 4 and 8 g kg⁻¹. Fish were fed to apparent satiation three times daily for 12 weeks under a 12 h light:12 h dark photoperiod and were weighed every two weeks following 24 h fasting and mild clove-oil sedation. Growth and feed-utilisation parameters, somatic indices, whole-body composition, haematological parameters, innate immune responses, and digestive and antioxidant enzyme activities were evaluated. Three fish were randomly sampled from each tank. Data were analysed by one-way analysis of variance followed by Tukey's multiple-comparison test, with significance accepted at $P < 0.05$.

Results

Diets containing bromelain-processed *Ulva* sp. significantly improved growth and feed-utilisation performance ($P < 0.05$). The highest final body weight (38.10 g), specific growth rate (2.41% day⁻¹) and protein efficiency ratio (1.99), together with the lowest feed conversion ratio (1.04), were recorded in fish fed 8 g kg⁻¹ bromelain-processed *Ulva* sp. The viscerosomatic index was reduced in the bromelain-processed groups and in the 4 g kg⁻¹ yeast-fermented group, while the mesenteric fat index was significantly lower at 4 and 8 g kg⁻¹ bromelain-processed *Ulva* sp. than in the control. Condition factor and hepatosomatic index were unaffected.

The highest red blood cell count, haemoglobin and haematocrit values were observed in fish fed 8 g kg⁻¹ bromelain-processed *Ulva* sp. This treatment also produced the highest protease, amylase and lipase activities. In contrast, the 2 g kg⁻¹ inclusion level of bromelain-processed *Ulva* sp. elicited the strongest responses in most innate immune indicators, including extracellular oxidative radical production, lysozyme, alternative complement pathway activity, bactericidal activity and phagocytic activity.

Discussion

The superior growth, feed utilisation and digestive enzyme activities observed with bromelain-processed *Ulva* sp. suggest that enzymatic pre-treatment enhanced nutrient accessibility and utilisation, consistent with improved digestive capacity and feed efficiency reported following bromelain supplementation (Wiszniewski et al., 2019; Qudratullah et al., 2025). The comparatively weaker responses to raw *Ulva* sp. indicate that restricted nutrient accessibility may limit its nutritional effectiveness, whereas processing can improve the digestibility and functional properties of macroalgal biomass (Wan et al., 2019). The stronger innate immune responses recorded at the 2 g kg⁻¹ inclusion level indicate a dose-dependent functional effect, consistent with evidence that seaweeds and their bioactive compounds can enhance fish immunity, although responses vary among species and dietary inclusion levels (Thépot et al., 2021). Overall, bromelain-processed *Ulva* sp. appears to be a promising functional ingredient for juvenile meagre, with the 8 g kg⁻¹ inclusion level favouring growth and digestive performance and the 2 g kg⁻¹ inclusion level eliciting the strongest innate immune responses.

Acknowledgment

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UTILIZING ADVANCED GENETIC IMPROVEMENT TOOLS TO MAXIMIZE RETURNS IN FIN FISH SPECIES

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Effective genetic improvement in finfish aquaculture begins with choosing the right breeding strategy, whether based on mass selection, family-based selection, or genomic selection. Each strategy varies in cost, complexity, and potential genetic gain, and selecting among them requires balancing breeding objectives, operational capacity, and expected commercial outcomes. Straightforward traits such as growth can be improved with simpler strategies, whereas complex traits - such as robustness, disease resistance, and correlated traits - benefit from more advanced strategies supported by modern genetic tools.

Within these strategies, genotyping, germ cell transfer (GCT), and genome editing (GE) serve as powerful tools to accelerate or enhance breeding outcomes. Genotyping enables accurate estimation of breeding values, improves selection intensity, and supports genomic selection programs for traits that are difficult or costly to measure. GCT offers a reproductive tool to boost efficiency by allowing sterilized surrogate hosts to produce donor-derived gametes. This is particularly useful for species with long generation intervals or challenging reproductive biology, enabling faster turnover of elite genetics and expanding breeding capacity. GE provides a precision tool that can introduce targeted modifications within a single generation, complementing the broader selective breeding strategy rather than replacing it.

Robust genetic progress ultimately relies on integrating phenotypic data, genotypic data, and operationally feasible breeding structures. This presentation evaluates how various genetic tools - genotyping technologies, germ cell transfer, and genome editing - can be embedded within well-designed breeding strategies to deliver sustainable, long-term improvements across multiple species. By aligning tools with strategy and commercial priorities, producers can build resilient breeding programs that strengthen husbandry foundations, enhance performance traits, and maximize returns on investment throughout the production cycle.

AN EGYPTIAN CASE STUDY ON THE IMPACT OF PISCIVOROUS BIRDS ON AQUACULTURE IN THE CONTEXT OF CLIMATE CHANGE

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Aquaculture is a vital economic and food-security sector in Egypt, yet its expansion has intensified conflicts with wildlife. This study presents the first comprehensive, multidisciplinary assessment of piscivorous bird impacts on coastal and brackish-water aquaculture in Port Said, Egypt. Through a 24-month mixed-methods case study integrating ornithological surveys, bioenergetics modeling and exclusion experiments, the research quantifies ecological and economic losses while situating the conflict within global change drivers. Key findings reveal that Little Egret (*Egretta garzetta*), Slender-billed Gull (*Chroicocephalus genei*), and Grey Heron (*Ardea cinerea*) dominate predation, causing direct economic losses averaging 8.7% of annual production value—equivalent to roughly EGP 11.25 million (USD ~360,000) annually for Port Said. Indirect impacts, including elevated fish stress, disease risk, and infrastructure damage, further compound losses. Farmer surveys indicate that current deterrents are largely ineffective and that bird predation ranks among the top challenges faced. The conflict is exacerbated by climate change, wetland degradation, and overfishing, which collectively increase system vulnerability. The study concludes by proposing an Integrated Bird Management (IBM) framework to promote sustainable coexistence, emphasizing physical exclusion, modern non-lethal deterrents, improved farm practices, and supportive policy measures. This work provides an empirical foundation for managing human-wildlife conflicts in aquaculture under changing environmental conditions.

THE IMPACT OF PISCIVOROUS BIRDS ON AQUACULTURE IN THE CONTEXT OF CLIMATE CHANGE: AN EGYPTIAN CASE STUDY

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Introduction

Aquaculture is a cornerstone of food security in Egypt, the largest producer in Africa, yet its expansion along the Mediterranean coast has intensified conflicts with piscivorous birds. Despite the global recognition of bird predation as a major economic constraint—with documented losses exceeding 30% of production in some regions—Egypt lacks empirical data on this conflict. The northern Delta lakes, including Lake Manzala adjacent to Port Said, are critical wetlands along the African-Eurasian flyway, hosting millions of migratory waterbirds. Concurrently, climate change, wetland degradation, and overfishing are eroding natural prey bases, potentially forcing birds toward aquaculture ponds. This study aims to conduct the first comprehensive assessment of piscivorous bird impacts on brackish-water aquaculture in Port Said, quantifying species-specific predation, economic losses, and the efficacy of current mitigation strategies within the context of global environmental change.

Materials and Methods

A 24-month mixed-methods case study (January 2024–December 2025) was conducted across 35 aquaculture farms in Port Said Governorate. Systematic ornithological surveys (point counts, transects, and 520 hours of focal behavioral sampling) were performed to characterize bird communities and foraging strategies. Exclusion experiments compared fish survival between netted ($n=15$) and open ponds. Bioenergetics modeling estimated consumption rates, while production records from 10-hectare seabass (*Dicentrarchus labrax*) operations quantified economic losses. Farmer interviews ($n=50$) assessed perceptions, mitigation costs, and management challenges. Spatial analysis using GIS mapped bird distribution relative to Lake Manzala.

Results

Thirty-three piscivorous bird species were documented, with three species dominating predation: Little Egret (*Egretta garzetta*) (28.5% of observations), Slender-billed Gull (*Chroicocephalus genei*) (18.7%), and Grey Heron (*Ardea cinerea*) (9.1%). Winter congregations reached exceptional densities: 48,266 birds at Lake Manzala North East and 28,555 at Port Fouad El Malaha. Direct economic losses averaged 12.7% of stocked biomass per cycle (range: 5–31%), equivalent to EGP 148,750 (USD ~4,950) per hectare per cycle. For a 10-hectare seabass operation, feeding-time predation alone caused seasonal losses of EGP 10,800–27,000. Extrapolated regionally, total annual losses reach approximately EGP 11.25 million (USD ~360,000). Indirect impacts included 220% higher cortisol levels and a 15.7% reduction in growth. Current deterrents were largely ineffective (mean efficacy 2.8/5); 65% of farmers admitted to lethal control despite legal protections.

Discussion

This study provides the first empirical evidence that piscivorous birds cause substantial economic losses to Egyptian aquaculture, with impacts exacerbated by climate-driven degradation of natural habitats. The convergence of migratory birds at aquaculture ponds reflects broader ecosystem stress—birds are increasingly ‘squeezed’ between a degraded Lake Manzala and an overfished Mediterranean Sea. The identification of species-specific foraging strategies and risk classifications enables targeted management. Cost-benefit analysis reveals that targeted netting of feeding areas (3.6-year payback) and automated laser systems (3.2-year payback) offer economically viable alternatives to current ineffective practices. These findings highlight the need for an Integrated Bird Management framework balancing aquaculture productivity with avian conservation obligations under Egyptian Law 4/1994 and international agreements.

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GENETIC PARAMETERS AND COMPARATIVE STRATEGIES FOR GENETIC IMPROVEMENT OF TAIL YIELD AND REDUCTION OF WATER LOSS DURING COOKING IN SHRIMPS: A CASE STUDY ON THE PACIFIC BLUE SHRIMP *Litopenaeus stylirostris*

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Introduction and objectives

The genetic improvement of the ratio of the edible part in shrimps is presently not undertaken in commercial breeding programs (Ren et al., 2025). We aim to fill this gap by estimating genetic parameters of abdominal yields and morphological predictors of those in the Pacific blue shrimp (*Penaeus stylirostris*) based on lengths measured by electronic caliper or on digital images, also integrating water loss after cooking. With these parameters, we estimate expected genetic gains on abdominal yields by sib-based selection or by mass selection on the predictor.

Material and methods

A total of 49 full and half-sib families were created by inseminating each dam with spermatophores from 2 sires (Haffray et al. 2025 and 2026). A sample of 1200 progenies were assigned to their parents with SNP markers and phenotyped at commercial size (≈ 14.5 g) by measuring body length and abdominal length on uncooked individuals, either with an electronic caliper or by computer vision from 2D pictures. The whole shrimps were weighted uncooked or after cooking by boiling, and abdomen weight was measured after cooking. We calculated the ratio of the cooked abdominal weight either to the uncooked body weight or to the cooked body weight. The water loss ratio after cooking was also calculated as the difference between uncooked and cooked body weights divided by uncooked body weight. We estimated genetic parameters for these traits and genetic gains obtained either by sib-based selection on tail yield or mass selection on the residual of the regression of abdominal length to total length.

Results

Heritability estimates were intermediate for the ratio of the cooked abdominal weight to the raw or cooked body weights (0.33-0.24), and lower when considered as residuals of the numerators to the denominators (0.20 and 0.12). The heritability of the ratio of abdominal length to total length increased when using computer vision instead of the electronic caliper (0.20 vs 0.07). The genetic correlations between predictors and the yields or residuals to predict were high (0.67-0.94) and higher when the predictors were measured by computer vision.

The simulated genetic gains per generation were similar between sib-selection and mass selection at similar selection pressures and increased with the increased selection pressure permitted by mass selection (10-30 %), compared to lower selection pressure possible by sib-selection (30-50 %).

Conclusions and perspectives

These results show it is possible to genetically improve abdominal yield by mass or by sib selection in the Pacific blue shrimps. The short interval of generation in shrimp is favorable for it progressive and slow improvement to better characterize potential impacts on other traits or relative organs development. The simple and highly genetically correlated indirect predictor evaluated is promising and future advances in 3D vision and AI may further improve phenotyping accuracy and increase heritability of future predictors. The negative genetic correlation between water loss at cooking and uncooked abdominal yield is also paving the way to better characterize the interaction between improvement of yields and flesh quality traits.

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ORIGIN AND FATE OF LONG-CHAIN POLYUNSATURATED FATTY ACIDS IN REPRODUCTION OF FEMALE *Hediste diversicolor* (OF MÜLLER, 1776) (ANNELIDA: NEREIDIDAE)

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Introduction

Meeting the aquatic protein demands of a growing global population necessitates increased aquaculture production. In Norway, further expansion of the salmon aquaculture industry depends on identifying alternative sources of marine proteins and lipids that support animal welfare. Polychaete biomass have emerged as promising substitutes for fish meal and fish oil in aquafeeds. Notably, *Hediste diversicolor* has demonstrated the ability to biosynthesize long-chain (C20-24) polyunsaturated fatty acids (LC-PUFAs) de novo, positioning it as an ideal candidate for converting low-value side streams into high-quality biomass within circular value chains. We hypothesize that the biosynthetic capacity of *H. diversicolor* to de novo synthesize LC-PUFAs is linked to the role of docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3) in oogenesis and reproduction: in conditions of limited dietary LC-PUFAs, endogenous synthesis may compensate, ensuring sufficient allocation of these lipids to oogenesis and spermatogenesis. In this 200-day experiment, we fed immature *H. diversicolor* a PUFA-deficient diet spiked with ¹³C-labelled palmitic acid (C16:0), a potential elongation/desaturation precursor, to test whether the worms biosynthesize EPA and DHA for maturation and whether this depends on dietary input.

Materials and Methods

Juveniles of *H. diversicolor* were collected from mudflats of Leangen Bay (63°26'24.5"N, 10°28'27.7"E) in the intertidal zone at low tide (as described in Machado et al., 2024). Red-colored, immature individuals (73.8±46.5 mg WW; n=48) of unknown sex were randomly distributed in transparent, cubical boxes (10 x 10 x 10 cm L x W x H; n=6) filled with a 5 cm thick layer of sand, supplied with continuous water flow (34 ppt salinity; 10 °C), using a 16h:8h light:dark cycle. The polychaetes were fed 50 mg of a LC-PUFA deficient diet spiked with ¹³C palmitic acid (16:0) tank⁻¹, three times per week, over a period of 200 days, in triplicates. Growth, survival, gamete development, fatty acid (FA) content and composition, and compound-specific stable isotope analysis (CSIA) of fatty acids of body tissues (somatic tissue) and gametes (reproductive tissue), were assessed. All statistical analyses were conducted using the inbuilt statistical package in SigmaPlot for Windows v15.0 (Systat Software, Inc., USA) with a significance level set to $\alpha=0.05$. Percentage data from FA composition analysis were arcsine-transformed prior to analyses.

Results

The male to female ratio was 5:1, 5:2 and 2:4, and the survival was 79.2±7.2 %, with no differences between replicates. The final weight (WW) of the polychaetes was 0.18±0.09 g (n=19). The SGR was calculated to 0.0039±0.0025. There was no correlation between oocyte size and polychaete weight ($r^2=0.07$; Non-linear Regression Analysis) and the average oocyte diameter was 180.2 ± 20.1 µm upon sampling. The quantitative FA analyses (mg/g DW) of juveniles, mature oocytes and somatic tissue of adult females collected from the wild, showed that the reproductive tissues were significantly higher in sum FA, sum SFA, sum MUFA, sum n-3 and sum n-6 than both full-body juveniles (One-Way ANOVA, Holm-Sidak post hoc test; $P = 0.001 - 0.026$) and adult somatic tissue (One-Way ANOVA, Holm-Sidak post hoc test; $P < 0.001 - 0.026$), whereas there were no differences for these groups of FAs between juveniles and adult somatic tissue, except for the sum of MUFAs and the sum of n-6 (One-Way ANOVA, Holm-Sidak post hoc test; $P = 0.025$). CSIA of female *H. diversicolor* reared on a LC-PUFA deficient diet containing ¹³C enriched 16:0 revealed that most of the detected FAs showed enrichment of ¹³C, including EPA and DHA, demonstrated by a positive $\delta^{13}\text{C}$ (‰) signal, with the exception of linoleic acid (18:2n6), α -linolenic acid (18:3n3), eicosadienoic acid (20:2n6) and dihomogamma-linolenic acid (20:3n6).

Discussion

This study demonstrated for the first time that ¹³C from spiked palmitic acid (16:0) is incorporated into EPA and DHA in adult female *H. diversicolor* somatic and reproductive tissues. The strongest ¹³C signal for DHA was detected in the oocytes, indicating active incorporation of dietary carbon into reproductive DHA. These findings support a link between LC-PUFA allocation and reproductive processes in this species, while the relative contributions of endogenous synthesis and microbial sources remain to be clarified.

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Acknowledgment

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ADVANCES IN AQUACULTURAL ROBOTICS AND AUTOMATION

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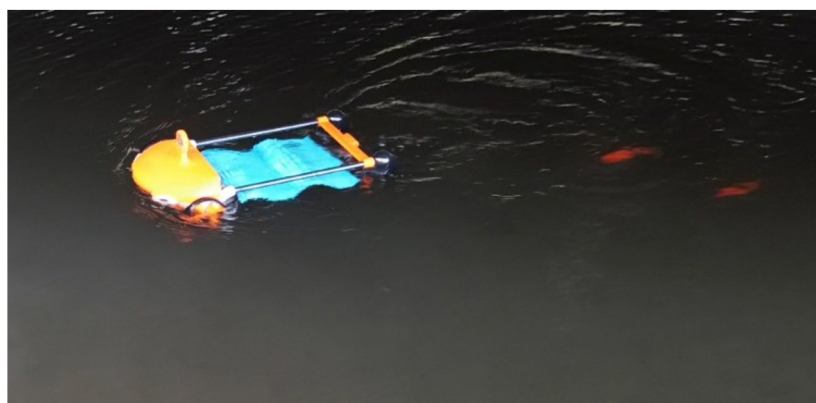
Aquaculture continues to expand as the fastest growing protein sector. As expansion continues, the need for and opportunity for cost effective automated and robotic solutions. This talk will summarize work completed and ongoing work to advance aquaculture robotics.

Three robotic approaches for aquaculture will be discussed. Surface vehicles with RC approach can provide easy access for operators to gather data on water quality, add feed or water quality components, remove mortalities or identify issues. In some cases, these can be placed in autonomous modes to rove or navigate to desired locations to assess or service aquacultured fish or equipment. Fleets of these vehicles can coordinate to do more complex tasks. Finally, unique single axis robots can operate in amphibious mode, allowing additional applications and opportunities.

We will review advances with aquabots, with focus on how smart systems can reduce challenges and add value. Among the applications are commercially available cameras and water quality sensors (e.g. salinity, temperature, dissolved oxygen). These devices can be mounted on autonomous or semi-autonomous aerial, surface and underwater vehicles to help sense water quality parameters in open (e.g. estuary, ocean) systems. They may also be used in culture systems (ponds, tanks, raceways) for diverse activities including feeding, harvest, waste and water quality management. With further development, these systems may also allow active interventions to reduce the impacts of storm events, toxicity and other environmental impacts, or to enhance productivity and sustainability.

Optimal management of these sectors may allow increased aquacultural productivity as well as optimal focus on conservation of habitat and species. Work has included a focus on smart thinking to add value; corobotics – i.e. robots providing humans with improved information, allowing wiser decisions. This work seeks to aid in developing more sustainable and productive aquaculture wisely using ethical robotics and automation. We will also discuss some near future applications of aquacultural robotics.

Figure 1: Novel single axis aqua-bot navigating a fish pond.



CHRONIC WARMING INCREASES PHYSIOLOGICAL DEMAND, ALTERS GONADAL CONDITION AND SUPPRESSES REPRODUCTION IN BLUE GOURAMI *Trichopodus trichopterus*

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Introduction

Rising freshwater temperatures can increase physiological demand while constraining reproduction in tropical fishes. Blue gourami *Trichopodus trichopterus* is a bubble-nesting ornamental species whose reproductive success requires coordinated gonadal development, nest construction, spawning behaviour and fertilisation. We tested how 120 days at 32 and 34 °C affected physiological demand, reproductive performance and broodstock gonadal condition relative to 28 °C.

Materials and Methods

Adult breeding pairs were maintained at 28, 32 or 34 °C. Spawning frequency was assessed in nine pairs per temperature; egg production and fertilisation were quantified from successful spawnings at 28 °C (n = 15) and 32 °C (n = 9). Pair-level feed intake (n = 9 per temperature) and individual opercular ventilation rate (n = 10 per temperature) were analysed by one-way analysis of variance. Gonadal histology was evaluated qualitatively (n = 2, 5 and 3 fish at 28, 32 and 34 °C); variable preservation precluded formal lesion or atresia scoring.

Results and Discussion

Spawning frequency declined from 2.44 ± 1.13 spawnings pair⁻¹ at 28 °C to 1.44 ± 0.88 at 32 °C and 0 at 34 °C ($F = 19.84$, $p < 0.001$; all pairwise comparisons significant). Mean egg production fell from 1806 ± 219.5 to 1236 ± 289.7 eggs, and fertilisation from $84.20 \pm 8.34\%$ to $68.37 \pm 6.73\%$ at 28 and 32 °C, respectively (both $p < 0.05$); no eggs were produced at 34 °C. In contrast, feed intake nearly doubled at 32–34 °C (28.46 ± 1.55 vs 51.09 ± 3.36 and 51.94 ± 2.59 g pair⁻¹; $F = 234.77$, $p < 0.001$), while ventilation increased from 71.4 ± 19.73 to 112.8 ± 11.40 and 111.0 ± 7.85 opercular movements min⁻¹ ($F = 28.28$, $p < 0.001$). Histology showed continued ovarian and testicular development at 32 °C. Notably, some 34 °C fish retained advanced secondary-growth/vitellogenic oocytes or active spermatogenic tissue despite complete absence of bubble nests and spawning. Thus, reproductive failure at 34 °C was not explained by universal arrest of gametogenesis, suggesting disruption of later processes linking gonadal development to reproductive behaviour and gamete release.

Conclusion

Chronic warming produced a graded reproductive decline. Exposure to 32 °C remained functional but suboptimal, whereas 34 °C exceeded the functional reproductive tolerance of broodstock despite retained advanced gametogenic stages in some fish. Reproduction therefore appears to be a sensitive endpoint for climate-resilient broodstock management.

Acknowledgment

This work forms part of the presenting author's doctoral research at Universiti Malaysia Terengganu.

AQUACULTURE-RELEVANT POLYSTYRENE MICROPLASTICS TRANSLOCATE TO GAMETES AND DISRUPT REDOX BALANCE WITHOUT IMPAIRING REPRODUCTIVE OUTPUT IN BROWN TROUT (*Salmo trutta*)

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Introduction and methods

Aquaculture organisms are exposed to microplastics (MPs) via feed formulated from contaminated fishmeal, packaging and the degradation of plastic infrastructure. Reported burdens of MPs in farmed salmonids range from $\sim 1.8 \times 10^3$ to 9.4×10^3 MPs fish⁻¹ per production cycle (Wang et al., 2022; Walkinshaw et al., 2022). However, most studies use unrealistically high concentrations and focus on marine species, thereby limiting their relevance to aquaculture. Most importantly, whether MPs translocate to gametes and impair reproductive performance under aquaculture-relevant exposure remains unresolved.

A total of 180 adult (3-year-old) *Salmo trutta* (570 ± 190 g; 38 ± 3 cm) were equally allocated to three treatments (~ 30 kg biomass tank⁻¹): control, low MPs (LMP; $\sim 4.8 \times 10^3$ MPs fish⁻¹), and high MPs (HMP; $\sim 4.9 \times 10^6$ MPs fish⁻¹). Fish were fed polystyrene microplastic (PS-MP)-spiked feed for 4 weeks (0.7–0.8% body mass day⁻¹), followed by commercial feed. Broodstock maturation, PS-MP translocation to gametes, antioxidant responses (superoxide dismutase [SOD], catalase [CAT], peroxidase [POD]), and reproductive outputs (semen volume, fecundity, hatching success) were assessed.

Results and discussion

PS-MPs were detected in both gametes (Fig. A2-3b–c), with higher particle numbers per gram of tissue and detection frequency in eggs than in semen (eggs: $0.53 \times 10^6 \pm 1.13$ PS-MPs g⁻¹; 4/10 fish; semen: $0.27 \times 10^6 \pm 0.59$ PS-MPs g⁻¹; 2/10 fish). In semen, only 1 μ m particles were detected, whereas eggs also contained 5 μ m; no 10 μ m particles were observed in either. These patterns indicate size-dependent translocation, likely driven by differences in gamete structure and retention capacity.

At the physiological level, PS-MPs disrupted antioxidant responses (Fig. d–f), with reduced enzyme activities (μ mol min⁻¹ g⁻¹ tissue) across tissues: semen SOD (Control: 9.76 ± 3.16 ; LMP: 6.79 ± 2.08 ; HMP: 6.59 ± 3.20), and egg CAT (2.35 ± 1.79 ; 0.21 ± 0.35 ; 0.03 ± 0.09) and POD (0.19 ± 0.34 ; 0.11 ± 0.34 ; 0.004 ± 0.004). As key enzymes that form the primary defence against reactive oxygen species, their suppression significantly reduces the ability of spermatozoa and oocytes to mitigate oxidative stress, potentially leading to increased lipid peroxidation, protein oxidation, and DNA damage.

On the other hand, reproductive parameters showed selective effects, with reduced maturation and spawning females in MP treatments (82.1% control vs 54.8% LMP and 45.9% HMP; Fig. g). While all males were spermiating at each assessment, semen volume was reduced by 40.2% in the LMP group compared to the control (Fig. h). However, fecundity (Control: 3505.3 ± 596.0 ; LMP: 3140.2 ± 1016.3 ; HMP: 3532.9 ± 663.6 ; Fig. i) and hatching success (Fig. j–k) did not differ among treatments, likely due to buffering by aquaculture practices.

These findings indicate that small MPs (≤ 1 μ m in sperm; ≤ 5 μ m in eggs) can translocate into gametes and disrupt antioxidant defences, contributing to oxidative stress and sex-specific reproductive effects, without altering overall reproductive output under aquaculture conditions. However, threshold-based analyses (Fig. 1h–k) reveal a higher proportion of broodfish with suboptimal reproductive outputs under MP exposure. Accordingly, salmonid aquaculture is unlikely to be constrained under standard hatchery management conditions, but these effects may become consequential in less controlled environments. In natural systems, where reproduction is unassisted and subject to multiple environmental stressors, such effects may translate into reduced recruitment. Given the increasing use of hatchery-reared fish for restocking, further work is needed to assess implications for wild populations.

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Figure 1. PS-MPs in *S. trutta*: Gamete translocation, burden, effects and reproductive outcome

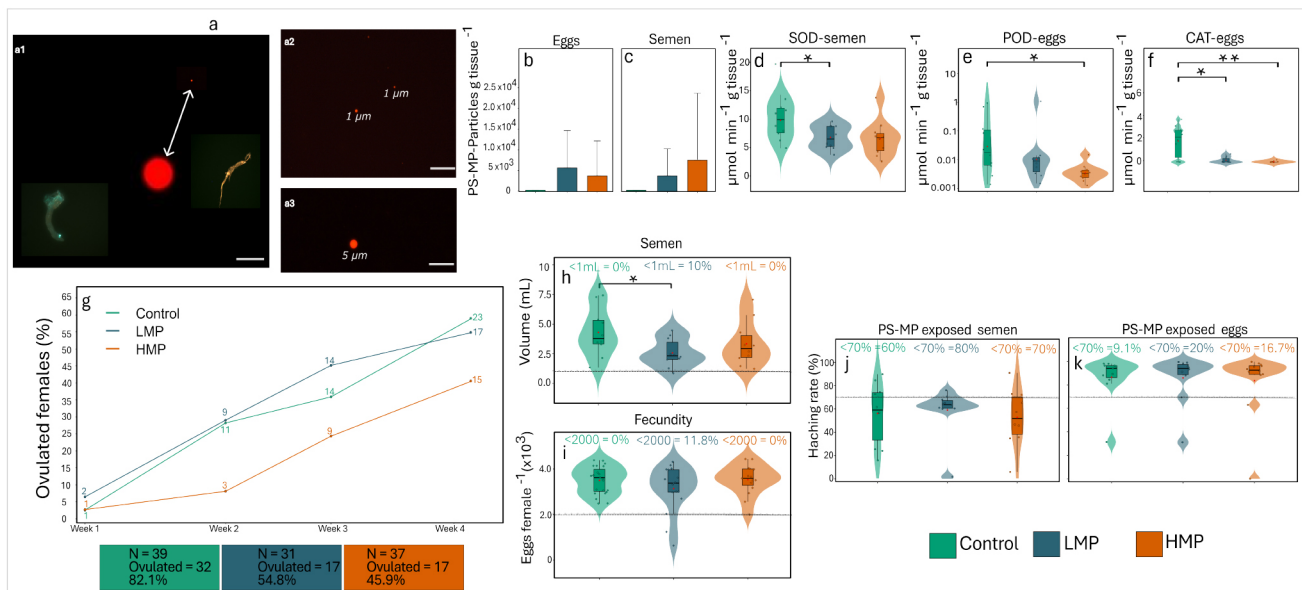


Figure 1. Effects of PS-MP exposure in *S. trutta*. Particle characterisation confirming spherical morphology and example of excluded non-spherical artefacts from counting (a); PS-MP abundance in gametes (b–c); antioxidant responses (d–f; $n = 10$ per treatment); POD in eggs plotted on a $\log_{10}$ scale for visualisation only; statistical analyses conducted on untransformed data); semen volume (h; $n = 10$ per treatment); fecundity (i; $n = 23, 17$, and 15 for Control, LMP, and HMP, respectively); and hatching success following PS-MP exposure in eggs and semen (j–k; $n = 10$ per treatment). Data are shown as violin plots overlaid with boxplots, individual observations, and means (red diamonds). Dashed lines denote functional failure thresholds (<1 mL semen volume, <2000 eggs per female, <70% hatch rate). For panels h–k, the proportion of individuals below the set threshold per treatment is indicated above each violin plot. Pairwise comparisons were performed among all treatments; only statistically significant differences are shown (* $P \leq 0.05$, ** $P \leq 0.01$).

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EFFICACY OF LIPOSOMAL NANOPARTICLE-BASED VIRAL HAEMORRHAGIC SEPTICEMIA (VHS) VIRUS ORAL VACCINE IN *Paralichthys olivaceus*

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Introduction

Viral hemorrhagic septicemia (VHS) causes serious economic loss in olive flounder aquaculture industry in Korea. Oral delivery provides a stress-free method of administration, making it ideal for mass immunization, particularly against infectious VHS, which poses a global threat to aquaculture.

Materials and Methods

In this study, we developed an optimal antigen delivery system using liposome encapsulating method for an oral VHS vaccine of olive flounder (*Paralichthys olivaceus*). Liposomes that encapsulate heat inactivated VHSV antigen, thereby forming microparticles for oral delivery (absorbed in feed) to flounder fingerlings. For the in vivo experiment, fish were injected with phosphate buffer saline (control, group 1) and heat inactivated VHSV (IP, group 2), were fed heat inactivated VHSV (oral, group 3), heat inactivated VHSV encapsulated liposome (uncoated liposome, group 4), heat inactivated VHSV encapsulated liposome and chitosan (coated liposome, group 5). We evaluated the antibody responses (via ELISA), hematological parameters, and the effects of vaccination on the growth and internal structures of flounder fingerlings.

Results

Our finding revealed that the antibody titer was highest at week 1 and that the chitosan coated liposome (group 5) showed a high antibody titer at week 2. Specific antibody titers measured on day 15 post-VHSV infection revealed that the oral liposome administration group exhibited the highest antibody titer. The lowest mortality rate was observed in the uncoated liposome VHSV vaccinated group (33%, group 4), followed by the chitosan-coated liposome group (40%, group 5).

Discussion

Our results suggest that oral administration of a chitosan-coated liposome vaccine represents a promising immunization strategy against VHSV. This method effectively represents a promising immunization strategy against VHSV. This approach effectively protects the viral antigen from digestive degradation, ensures ease of inoculation, and demonstrates excellent stability when administered orally, making it an effective approach for preventing viral hemorrhagic septicemia in farmed fish.

Acknowledgment

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OFFSHORE AQUACULTURE – TECHNICAL DEVELOPMENT OF A COMBINED AQUACULTURE IN THE WESTER BALTIC

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The offshore aquaculture project, which was submitted in 2023 and is funded by European and state funds, aims to develop combined aquaculture in the Baltic Sea. The aim is to promote the spread and economic viability of combined fish and shellfish farming in the Baltic Sea and to gain greater recognition for it. Furthermore, environmental compatibility and economic efficiency are to be evaluated in order to create aquaculture production in the western Baltic Sea off Mecklenburg Western Pomerania that is as sustainable, environmentally friendly, local, and accessible to all as possible. The overall project represents a new approach, as the difficult hydrodynamic and harsh conditions, characterized by high wind speeds and accompanying wave heights, vary greatly in the coastal region of the Baltic Sea, for example, from those in the fjords of Norway. For fish aquaculture, the target fish species is rainbow trout (*Oncorhynchus mykiss*). The target shellfish species that is produced for shellfish aquaculture is the blue mussel (*Mytilus edulis*). Other objectives of the project in the field of fish farming include two separate pen systems that are to be installed and tested in the western Baltic Sea and subsequently stocked with fish. One system, called “The Cage,” is a net-supported system; the other, called “Björn,” is a steel cage shaped like a truncated octahedron. Three different systems were designed and visualized for mussel farming. These include one underwater longline system and two surface longline systems, one of which is a double longline system and the other a single longline system with different types of buoys. In early 2024, all three shellfish systems, as well as the net support system “The Cage,” were designed, visualized, and then hand-built on land. In the end of 2024, all of the constructed systems were transported to the Nienhagen Fisheries Reserve, located in the western Baltic Sea not far from Rostock, and installed there for a tasting phase. In early 2025, two of the three installed mussel farms were lost due to severe winter storms; only the above-water double-line system could be saved. However, the first mussel harvest was lost due to the storms and predators. Because of the difficult conditions throughout 2025, only a single mussel farm was installed during the entire year and was harvested at the end of 2025. In mid-2025, the first fish were stocked into the net carrier system over a three-month period. However, due to predators, half of the stock was lost, so that by mid-2025 only half of the stock could be harvested. After the harvest, the net carriers were restocked with rainbow trout following a net change and harvested at the end of 2025. Both harvests yielded excellent results in terms of both fish biomass and mussel biomass. The total catch weighed approximately 209.1 kg, consisting of 417 trout, resulting in an average weight of 0.5 kg per fish. 7.2 kg of commercially viable mussels were harvested per meter of collector line. Extrapolated to the entire 450-meter double-longline system, this would amount to approximately 3,237 kg of commercially viable mussels. Other results such as fish index and mussel index, were also wide above average. In early 2026, all three mussel systems were reinstalled and equipped with data loggers that record axial movement. Thus, the mussel system with the least axial movement will be the most efficient in terms of losses and, consequently, the most useful and resilient under harsh conditions. For a future project we want to establish a community to pool their knowledge and expertise in order to produce a sustainable blue circular nutrient economy in the Baltic Sea area. Substituting fishmeal with regional mussel protein production to reduces pressure on wild stocks and closes the nutrient cycle, making regional offshore aquaculture circular.

LIGHT PERIOD MANIPULATION IN RAINBOW TROUT PRODUCTION INFLUENCES THE HORMONAL CONTROL OF GROWTH AND GROWTH IN BRACKISH WATER CAGES

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Introduction

In a combined RAS (recirculating aquaculture system) and sea cage farming, the fish are first farmed in a RAS and then transferred to sea cages. Using continuous light and feeding is a common practice in RAS. This helps maintain stable water quality, which is crucial for the fish health and the good performance. In contrast, the light conditions in sea cage farming follow natural diurnal and seasonal cycle, affecting fish physiology. For example, although continuous light can result in a higher growth rate in RAS, performance of salmonids farmed under continuous light has been unoptimal after they are transferred to sea (Hänninen et al., 2026; Ytrestøyl et al., 2023). Yet, there is lack of information if various light rhythms in RAS could prepare the rainbow trout (*Oncorhynchus mykiss*) for natural light in the open sea, and how this could affect the hormonal control of fish growth. Therefore, here we investigated 1) do growth and/or hormonal parameters of rainbow trout differ between different light and feeding rhythms in RAS, 2) do different light and feeding rhythms in RAS affect growth and/or hormonal parameters of rainbow trout in brackish water cages, and 3) do growth and/or hormonal parameters of rainbow trout differ between different light rhythms in brackish water cages.

Materials and Methods

Juvenile rainbow trout (7 g) were farmed in RAS at 15 °C under continuous light (LD24:0) and a light rhythm (LD18:6) for six months. The fish were then transferred to brackish water cages located in the Baltic Sea. An underwater light was installed in half of the cages to create light groups with continuous (CL) and natural light (NL). Thus, at the cages we had four groups: 1) LD24:0/CL, 2) LD24:0/NL, 3) LD18:6/CL, and 4) LD18:6/NL. The fish were farmed in the cages for one month to size of approximately 630 g. Sampling was carried out the day before the transfer and after one month in the cages. Length and weight were measured. Based on the fish growth data, the specific growth rate (SGR), the feed conversion ratio, and the condition factor were calculated. Blood samples were taken for haematocrit, haemoglobin, plasma vasotocin (VT), plasma growth hormone, and plasma insulin-like growth factor 1 analyses.

Results

In the first sampling (before transfer), shortening the photoperiod did not have a negative effect on fish growth or hormonal parameters in the RAS. In the second sampling (after transfer), the light rhythm (CL or NL) during the brackish water cage period significantly affected the SGR ($p < 0.001$). However, the light and feeding rhythm during the RAS period, or the interaction between the light rhythms during the RAS and brackish water cage periods, had no significant effect ($p = 0.626$ for both). The SGR was 1.13 ± 0.02 in the LD24:0/CL treatment, 1.02 ± 0.02 in the LD24:0/NL treatment, 1.13 ± 0.02 in the LD18:6/CL treatment, and 1.04 ± 0.02 in the LD18:6/NL treatment. Additionally, we observed that the light and feeding rhythm (LD24:0 or LD18:6) during the RAS period significantly affected the plasma VT levels ($p = 0.050$). Conversely, light rhythm during the brackish water cage period, or the interaction between the light rhythms during the RAS and brackish water cage periods, had no significant effect ($p = 0.108$, $p = 0.368$, respectively). Plasma VT levels were 32.0 ± 8.3 ng L⁻¹ in the LD24:0/CL treatment, 40.5 ± 10.2 ng L⁻¹ in the LD24:0/NL treatment, 47.0 ± 8.3 ng L⁻¹ in the LD18:6/CL treatment, and 73.6 ± 10.2 ng L⁻¹ in the LD18:6/NL treatment.

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Discussion

The fish grew equally well in both treatments (LD24:0 and LD18:6) in the RAS, despite previous studies have shown better growth under continuous light (Ytrestøyl et al., 2023). The discrepancy may be due to differences in the duration of the dark period used in the experiments. Light and feeding rhythms applied in the RAS did not affect the growth of rainbow trout in brackish water cage farming, and all groups were able to start growing in brackish water as compared to some previous studies where even negative growth have been detected (Hänninen et al., 2026). However, continuous light and feeding (LD24:0) during the RAS period seem to decrease plasma VT level compared to the light and feeding rhythm (LD18:6) in brackish water cage farming. VT regulates osmoregulation, as well as circadian and annual rhythms (Kulczykowska and Stolarski, 1996). VT levels were expected to behave in the opposite way, since they rise in response to stressors (Gilchriest et al., 2000). Continuous light during the brackish water period seems to increase the growth of rainbow trout, but it does not seem to affect their hormonal parameters. In conclusion, since the fish are transferred in spring when the days are naturally long, there is no need to acclimatise them to the natural light rhythm by adjusting light rhythm in the RAS. However, if the aim is to enhance fish growth in brackish water, providing a more consistent supply of light through underwater light in cages or delaying the transfer could be viable solutions.

Acknowledgment

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MARINE-DERIVED MINERALS AS A FUNCTIONAL FEED STRATEGY FOR *Litopenaeus vannamei*

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Introduction

Disease-related mortality remains a major challenge for intensive shrimp aquaculture, creating demand for functional feeds that support resilience without compromising production performance. Marine-derived ingredients provide bioavailable minerals and bioactive compounds with potential to support physiological and immune functions. This study evaluated a marine-derived mineral ingredient (Marisert) as a functional feed additive for Pacific white shrimp (*Litopenaeus vannamei*).

Materials and Methods

A 28-day 2 × 2 factorial feeding trial compared a control diet containing limestone with a diet containing 0.3% Marisert at 100 and 400 shrimp m⁻². Marisert contained calcium (29.5%), magnesium (5.7%) and an *Ascophyllum nodosum* fraction containing alginate, fucoidan, laminarin, mannitol and polyphenols. Growth, specific growth rate, feed conversion ratio, survival and hepatosomatic index were assessed. Shrimp were subsequently challenged with *Vibrio parahaemolyticus* causing acute hepatopancreatic necrosis disease (AHPND) and white spot syndrome virus (WSSV).

Results and Discussion

Marisert inclusion maintained zootechnical performance, with no significant differences in final weight, specific growth rate, feed conversion ratio or hepatosomatic index relative to controls ($P > 0.05$). Feed conversion ratios remained efficient across treatments (0.781–0.794). Stocking density significantly affected feeding-phase survival, demonstrating the production pressure associated with intensive culture. During the AHPND challenge, Marisert-fed shrimp showed a promising numerical improvement in response relative to corresponding controls, although dietary differences were not statistically significant. The challenge included 15 shrimp per treatment and therefore had limited statistical power. No clear beneficial effect was detected following WSSV challenge.

Conclusion

The study provides proof-of-concept for the potential efficacy of a marine-derived mineral ingredient as a functional shrimp feed additive. At only 0.3% inclusion, Marisert maintained growth and feed efficiency while showing a promising response during bacterial AHPND challenge. These attributes support further evaluation of marine-derived mineral supplementation as a nutritional strategy to improve shrimp resilience and address disease-related production losses in intensive aquaculture. Larger challenge trials are required to confirm the response and quantify its commercial relevance.

Treatment	Final weight (g)	FCR	Feeding survival (%)	AHPND survival (%)
Control 100 m ⁻²	5.17 ± 0.11	0.787 ± 0.019	98.0 ± 2.0	26.7
Marisert 100 m ⁻²	5.17 ± 0.13	0.788 ± 0.002	95.2 ± 6.6	46.7
Control 400 m ⁻²	5.05 ± 0.13	0.794 ± 0.021	67.6 ± 1.7	40.0
Marisert 400 m ⁻²	5.10 ± 0.08	0.781 ± 0.013	67.7 ± 1.6	66.7

Table 1. Growth performance and survival following the acute hepatopancreatic necrosis disease challenge. Feeding-trial values are mean ± standard error (n = 3).

NEW LIVE FEED FOR STARTFEEDING OF ATLANTIC COD (*Gadus morhua*) - BLUE MUSSEL EGGS AND BALANUS NAUPLIA

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Introduction

Codfarming in Norway is revitalized due to sharp declines in wild quotas and successful development of a selective breeding for Atlantic cod. Rearing of Atlantic cod (*Gadus morhua*) larvae relies heavily on the availability of suitable live feed that meets larval nutritional requirements while supporting efficient feeding behavior and survival. Rotifers (*Brachionus* spp.) and artemia (*Artemia salina*) are currently the standard first-feeding organism in cod larviculture, yet their small size, swimming behavior, and nutritional profile may limit larval growth and robustness during early developmental stages. Nauplii of the barnacle *Balanus* spp. represent a potential alternative or supplementary live feed, offering larger prey size, distinct motility patterns, and a naturally marine lipid composition that may better match the feeding ecology of cod larvae. This study evaluates the performance of *Balanus* nauplii and blue mussel eggs (*Mytilus edulis*) compared to rotifers and artemia as live feed, with particular emphasis on growth, survival during and deformities.

Materials and methods

Eggs and sperm were stripped from sixth-generation broodstock originating from the Norwegian National Cod Breeding Program and fertilized according to the program's standard protocol. Five full-sib families were produced, and fertilized eggs from each family were incubated separately in 25 L conical silo incubators supplied with flow-through ambient seawater at 4–5 °C. Quadruplicate tank design was used and the startfeeding tanks had 190 l volume. 15000 larvae per tank were used. Water temperature was then gradually increased from 4–5 °C to 10 °C over a period of 5–10 days (approximately 1 °C per day).

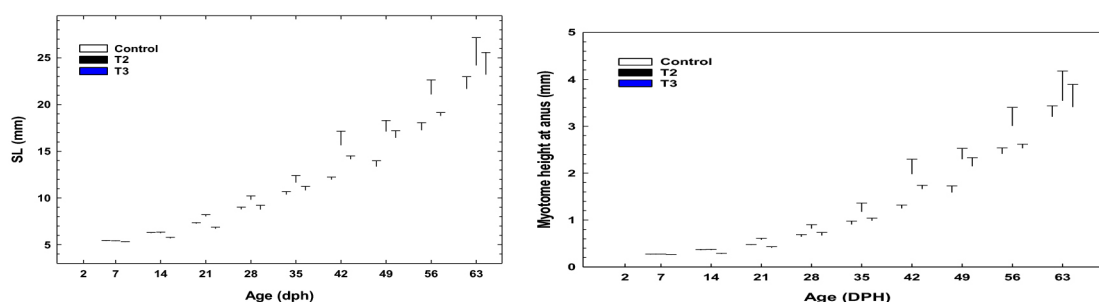
Three different startfeeding protocols were compared. Control 1 was the standard protocol at the National breeding program with rotifers and artemia and late weaning. Treatment 2 (T2) protocol 2 used a combination of rotifers and small *Balanus* nauplia (cryo S) and large *Balanus*(cryo-L) nauplia that replaced artemia and then weaning. Treatment 3 (T3) protocol used blue mussel (cryo Micro) eggs that replaced rotifers and large and small *Balanus* nauplia (Cryo S and Cryo L).

Deformities was measured with x-ray at 180 days post hatch.

Results

Protocol 2 gave higher growth rates, condition factor and myotome heights than protocol 1 and 3. Protocol 1 and 2 had the highest survival at 72 dph. Protocol 1 and 2 had lower vertebral deformities than protocol 3.

Growth. Standard Length (mm) and Myotome height for control, T2 and T3 related to days post hatch (dph).



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There were no significant differences between treatments in neck deformities. The control group had control group had lower rate of vertebra deformities than the other treatments. The control group also had lower rates of palatine bone deformities. Other deformities such as vertebrae fusion, lordosis, upper jaw/scull and lower jaw had minor differences between treatments, but severity differed.

Discussion

Balanus nauplia as live feed shows potential to replace traditional live feeds at least partly. These live feeds are also relatively early in their industrial development and improvements in products and protocols are most likely to happen. *Balanus nauplia* production is also interesting as live feed when it comes to securing local value chains in a rapidly changing world. The logistics of the product also raises possibilities for low carbon footprints related to transport. Further development of this new live feed in terms of quality, optimum protocols, carbon footprints, and local value chain security will be important in further research.

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CLOSING NUTRIENT CYCLES IN TRADITIONAL CARP POND AQUACULTURE: THE TEICHKREIS PROJECT

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Introduction

Traditional carp ponds in Bavaria (Germany) and South Bohemia (Czech Republic) represent one of Europe's oldest aquaculture systems and provide important ecological services beyond fish production, including biodiversity conservation, water retention, and cultural landscape preservation (Leibniz Institute of Freshwater Ecology and Inland Fisheries, 2023). However, these shallow pond ecosystems are increasingly affected by climate change, eutrophication, internal phosphorus loading, and declining dissolved oxygen concentrations during summer, resulting in reduced production efficiency and increasing management costs (Boyd, 2020). While conventional pond management practices, such as winter drainage, have historically mitigated nutrient accumulation, their effectiveness is decreasing due to warmer winters and altered hydrological conditions (Poschlo et al., 2026). The INTERREG-funded **Teichkreis** project investigates whether natural sorption materials can recover excess nutrients from pond water and whether nutrient-enriched materials can subsequently be recycled as agricultural fertilizers, thereby establishing a regional circular nutrient management strategy linking aquaculture and agriculture.

Materials and Methods

Teichkreis is a cross-border collaboration between Hof University of Applied Sciences (Germany) and the University of South Bohemia (Czech Republic), supported by regional aquaculture stakeholder organizations. Four natural sorption materials—zeolite, bentonite, expanded clay, and biochar—are evaluated for their capacity to adsorb ammonium and phosphate under conditions representative of Central European carp ponds. Laboratory batch adsorption experiments are conducted using water chemistry derived from Bavarian and Bohemian pond systems, followed by ecotoxicological assessments to verify fish compatibility. The best-performing materials are subsequently investigated in flow-through column experiments to evaluate adsorption kinetics and breakthrough behaviour under simulated hydraulic conditions. Final validation will be performed under commercial pond farming conditions to assess operational feasibility and nutrient recovery potential. Nutrient-loaded sorption materials will additionally be evaluated for their suitability as recycled fertilizers in agricultural production.

Results

The project is currently progressing through laboratory-scale investigations. Water quality surveys conducted in representative Bavarian and Czech pond systems have established the environmental conditions for experimental testing. Initial work focuses on quantifying nutrient adsorption performance, evaluating material stability, and identifying sorption media suitable for practical application without adverse effects on fish welfare. Early stakeholder engagement involving regional pond farmers confirmed nutrient accumulation, internal phosphorus loading, and seasonal oxygen depletion as major production constraints (Kowalczywska-Madura et al., 2018). The discussions also highlighted strong interest in practical nutrient recovery technologies that can be integrated into existing pond management practices.

Discussion

Teichkreis applies a circular economy approach to aquaculture by considering surplus nitrogen and phosphorus as recoverable resources rather than waste. Instead of continuously removing nutrients throughout the production cycle, sorption materials are intended for use during management events associated with unavoidable nutrient losses, including pond drainage, harvesting, overflow events, and sediment removal. This strategy aims to improve water quality while maintaining pond productivity and recycling recovered nutrients back into regional agricultural systems. By integrating environmental engineering, aquaculture research, and stakeholder participation, the project seeks to develop scientifically validated and practically applicable recommendations for sorption-based nutrient management in traditional pond aquaculture. The outcomes are expected to contribute to climate-resilient pond management and enhanced sustainability of Central European carp farming.

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Acknowledgment

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FROM SHEEP TO FOOD: SUSTAINABLE GROWING SUBSTRATES FOR AQUAPONIC CROP PRODUCTION – THE NAWARUN PROJECT

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Introduction

Sustainable aquaponic production requires growing media that efficiently regulate water availability, nutrient retention and root-zone conditions while minimizing environmental impacts (Abdul Hamid et al. 2022; Fang et al. 2017). Bio-based materials offer a promising alternative to conventional substrates because they provide physical support, enhance nutrient storage, and contribute to circular-economy principles (Gruda 2019). Among these materials, sheep wool is an underutilized agricultural by-product with high water-holding capacity, high organic matter content, and the potential to act as a slow-release nutrient source (Kovács et al. 2025). However, the suitability of sheep wool-based substrates for seedling production in aquaponic systems has received little attention. Therefore, this study compared rockwool with four substrate formulations containing different proportions of sheep wool and viscose fibers. Tomato, lettuce, and kohlrabi were cultivated in a recirculating aquaponic system, and seed germination, plant growth, and biomass production were evaluated to identify sustainable alternatives to conventional growing media.

Materials and Methods

The experiment was conducted under two growing conditions: a recirculating aquaponic system and a rack-based cultivation system irrigated with tap water. Five substrate treatments were tested, including rockwool as the control and four formulations containing different proportions of sheep wool and viscose fibers. The performance of the substrates was evaluated using tomato (*Solanum lycopersicum* L.), lettuce (*Lactuca sativa* L.), and kohlrabi (*Brassica oleracea* var. *gongylodes*). Seeds were directly sown into substrate plugs and cultivated under the respective growing conditions. Germination, plant survival, shoot growth, root development, fresh biomass, and dry biomass were assessed. Data were analyzed using factorial ANOVA to evaluate the effects of substrate treatment, growing condition, plant species, and their interactions. Statistical significance was accepted at $P < 0.05$.

Results

The study is currently in progress, with experiments being conducted under both aquaponic and conventional rack-based cultivation conditions. Current work focuses on evaluating seed germination, seedling establishment, and early plant growth across different substrate formulations and crop species. In addition, the physical performance of the substrates, including structural stability and water retention, is being assessed to identify formulations suitable for practical application in aquaponic production systems. The generated data will provide the basis for selecting bio-based substrates with the greatest potential to replace conventional rockwool.

Discussion

This study explores the potential of sheep wool waste as a sustainable alternative to conventional rockwool in aquaponic seedling production. Sheep wool, an underutilized agricultural by-product, represents a promising approach to valorising biological waste streams and reducing dependence on mineral-based substrates. The use of these materials supports circular economy principles by transforming agricultural residues into value-added products for aquaponic cultivation (Camilli et al. 2025). In addition, biodegradable substrates may improve the environmental sustainability of aquaponic systems and contribute to more resource-efficient food production (Komorowska et al. 2023). The findings are expected to provide practical guidance for integrating sustainable growing media into future aquaponic and soilless cultivation systems.

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EXPLORING THE CAPACITIES OF *Enchytraeus albidus* FOR OMEGA-3 LC-PUFA BIOSYNTHESIS: IMPLICATIONS IN AQUACULTURE

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Introduction

Many aquatic invertebrates, including mollusks, crustaceans, cnidarians and annelids, may serve as exceptional sources of the essential nutrients, including the long-chain ($\geq C20$) polyunsaturated fatty acids (LC-PUFAs), namely arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3). While some of the LC-PUFAs contained in invertebrate body lipids derive from dietary input, some aquatic invertebrates have the capacity to inherently synthesize LC-PUFAs from adequate short-chain precursors (Monroig et al., 2022) thanks to the presence of complementary set of enzymes called elongases (Elovl), front-end desaturases (Fed) and methyl-end desaturases (ω x). Polychaetes are arguably the most studied group within the Annelida phylum regarding their LC-PUFA biosynthetic capacity; however, other groups, such as oligochaetes, have been largely overlooked. Focusing on the terrestrial oligochaete *Enchytraeus albidus*, an organism often used as live feed and functional feed ingredient in fish larviculture (Grantland et al., 2025), the present study aims to determine the capacity of *E. albidus* to synthesize LC-PUFAs through the identification and characterization of its enzymatic repertoire of fatty acid elongases and desaturases.

Materials and Methods

To identify the potential elongases (Elovl) and desaturases (Fed and ω x), tblastn searches were conducted against publicly available Transcriptome Shotgun Assembly (TSA) databases from *E. albidus*, using as query known sequences of desaturases and elongases from marine polychaetes. After sequence validation, the putative elongase and desaturase sequences retrieved from *E. albidus* were used as a reference to design primers enabling the amplification of the complete open reading frames (ORFs) by PCR. The PCR template was cDNA prepared from total RNA from fresh worms (pools of three individuals). The PCR products corresponding to the ORFs of the putative elongases and desaturases from *E. albidus* were 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 encoded enzymes, we compared the deduced protein sequences with other annelid elongases and desaturases, identified conserved domains, and performed a Maximum likelihood phylogenetic tree along with known sequences from multiple invertebrates. For functional characterization, transgenic yeast transformed with the pYES2 constructs containing the *E. albidus* ORF of desaturases and elongases were supplemented with potential fatty acid substrates selected based on currently established LC-PUFA biosynthetic pathways (Monroig et al., 2022). After 48 h, yeast cultures were harvested, and total lipids were extracted for further derivatization into fatty acid methyl esters (FAME). FAMES were analyzed through gas chromatography and mass spectrometry detection (GC-MS), and conversions were calculated using the formula $[\text{product area} / (\text{product area} + \text{substrate area})] \times 100$.

Results and Discussion

Three Elovl, two Fed and two ω x with putative roles in the LC-PUFA biosynthesis were identified in *E. albidus*. Both the elongases and desaturases contained histidine boxes and conserved domains expected for their respective protein family (Hashimoto et al., 2008). The phylogenetic analyses of the three *E. albidus* Elovl sequences clustered closely with well-described elongases from other invertebrates, including annelids, allowing us to classify them as Elovl2/5, Elovl4 and Elovl8. The two *E. albidus* Fed sequences, namely Fed1 and Fed2, were clustered in the so-called “Group A” and “Group B”, respectively. The *E. albidus* ω x desaturases, termed hereafter as ω x1 and ω x2, clustered closely to other methyl-end desaturases from Annelida, within Clade 3 as referred to by Kabeya et al. (2018). Concerning the functional assays, yeast expressing the three *E. albidus* elongases showed activity towards C18 and C20 fatty acid precursors. Regarding desaturases, the *E. albidus* Fed1 exhibited $\Delta 5$ desaturation activity, being able to produce LC-PUFAs like ARA and EPA, while Fed2 showed a combination of $\Delta 6$ and $\Delta 8$ activities. These newfound enzymes displayed similar functions to their homologs found in polychaetes such as *Hediste diversicolor* (Villena-Rodríguez et al., 2025). The assays on the *E. albidus* methyl-end desaturases showed complementary functions. Yeast expressing the *E. albidus* ω x1 was able to convert oleic acid (18:1n-9) into linoleic acid (18:2n-6), confirming this enzyme is a $\Delta 12$ desaturase. The ω x2 showed $\Delta 15$, $\Delta 17$ and

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$\Delta 19$ activities, converting a variety of omega-6 substrates into omega-3 LC-PUFAs (e.g., EPA), and exhibiting analogous activities to other methyl-end desaturases of polychaetes like *H. diversicolor* (Kabeya et al., 2020). Collectively, the present investigation demonstrates that *E. albidus* has the complete enzymatic repertoire needed for the *de novo* biosynthesis of LC-PUFAs including ARA and EPA, thus allowing the production of live prey with high nutritional value and eliminating the requirement for exogenous enrichment prior to use as larval feed.

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MODULAR PATHWAYS FOR SUSTAINABLE AQUAFEED DRYING

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Introduction

Drying typically accounts for more than 60% of the energy demand in aquafeed extrusion, and for most facilities this energy still comes from fossil fuels. As feed mills face pressure to improve sustainability and emissions in aquaculture and prepare for possible carbon regulations, aquatic feed dryers become a natural starting point for decarbonization efforts.

Past attempts at heat recovery have been limited by two persistent challenges: (A) exhaust air is hot, humid, and dust-laden, causing fouling and performance decline in heat exchangers; and (B) conventional energy sinks, mainly dryer make-up air, represent only 10–15% of the dryer’s thermal load due to the main load deployed onto recirculated air inside dryers. To unlock larger savings, both of these source and -sink side challenges must be addressed.

This paper presents a modular approach enabling aquafeed producers to increase heat recovery today while preparing for hybrid or fully electrified dryer operation in the future.

Modular Heat Recovery Pathways and Exhaust Air Pre-Treatment

The challenges of dust-laden air, fouling and lack of heat sinks must be addressed for successful decarbonization of aquatic feed and improving sustainability in aquaculture in general. A dedicated Heat Recovery Unit (HRU) is introduced here. The HRU combines exhaust air cleaning and coil area into a single modular system that can be expanded over time. At the HRU inlet, a rotating drum-filter with suction nozzles removes fine particles from the wet exhaust stream. This step prevents fouling of the heat exchanger coils, stabilizes heat transfer performance, and maintains consistent back pressure towards the dryer. Once the air is cleaned, the HRU can transfer energy into one or more sinks depending on the different integration pathways:

- Preheating dryers make-up air or process water. This provides a straightforward energy efficiency improvement and typically recovers 10-15 % of dryer energy.
- Preheating process water for the feed manufacturing process, including boiler feedwater
- Building- and facility heating,
- Auxiliary water heating, e.g. cleaning water.
- With suitable HRU sizing, integration with high-temperature heat pumps to get to temperatures that allows heating of air *internal* to the dryer. This hybrid approach enables substantial reductions in fossil fuel, and supports future electrification using natural refrigerants

Because the HRU is modular, aquafeed producers can adopt the level of integration that matches their site’s energy demands and investment horizon, starting with basic heat recovery and progressing toward partial or full electrification when conditions are favorable.

Table 1. Example numbers for HRU systems as well as for its integration with the hybrid dryer using high-temperature heat pumps using natural refrigerants.

Dryer @ 1.6 MW 10 tons/h feed 2.1 tons H ₂ O/h	Heat recuperation (HRU)		Hybrid dryer integration
	1 coil bank	2 coil banks	2 coil banks
Particle fines removal	F7	F7	F7
Odor reduction	+	++	+++
Water recovery estim. [kg/h]	350	500	900
Energy recovery [% dryer load]	28 %	53 %	64 %
To dryer [kW]	200	200	1600
Process liquids [kW]	250	250	
Aux. heating [kW]	-	400	
Electrical power [kW]	19	21+	570
ROI [years]	1.5-2.5	1-2	4 - 8

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Figure 1. ROI analysis based on a simulation for the HRU 1-coil solution in Western Europe

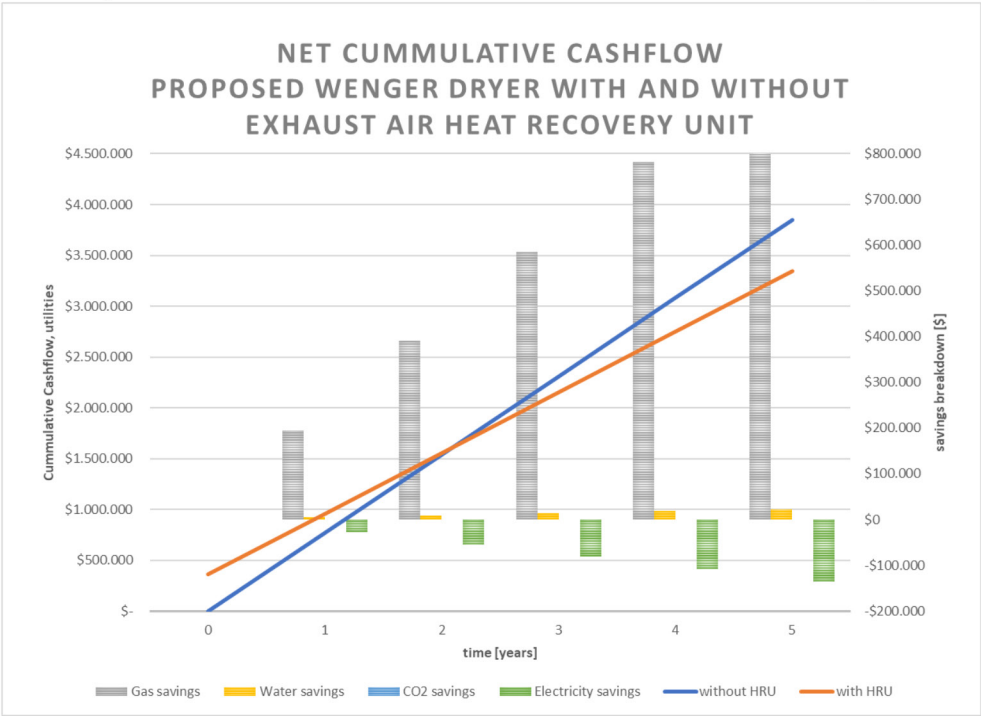
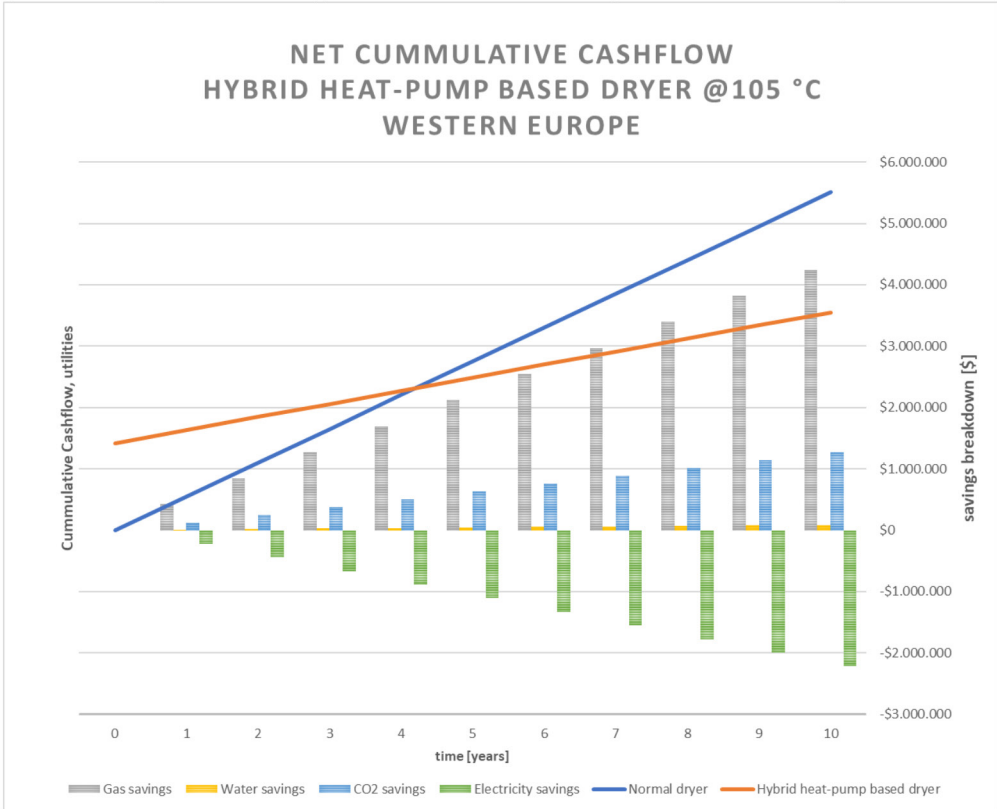


Figure 2. ROI analysis based on a simulation for integration level using hybrid high-temperature heat pump dryer using natural refrigerants and coupled to the dual-coil HRU system. Western Europe.



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Performance Outcomes and Decarbonization Potential

The integrated filtration and HRU system significantly increase the recoverable portion of dryer energy, with 28-53% recovery depending on coil configuration. When combined with a high-temperature heat pump using a natural refrigerant, the system can cut energy use by up to 64% and operate with up to 100% electric heat input, offering a practical pathway toward decarbonization of feed for aquaculture.

In parallel, fine-particle removal ($\eta_{F7} \approx 65\%$ of particles below $2.5 \mu\text{m}$) improves air cleanliness and reduces odor, which can be valuable for aquaculture feed plants located near communities or sensitive environments. Water condensation from the exhaust stream also yields 350–900 kg/h of recoverable water, depending on configuration.

Economic modeling shows a solid commercial foundation for HRU-only systems (1-2 years) and medium term and tangible ROIs for hybrid dryer heat pump integration, depending on regional energy prices. The overall pathway enables both immediate savings and long-term readiness for high-efficiency, low-carbon aquafeed production.

Performances for energy recuperation and decarbonization aspects of the different integration levels / solutions is seen in Table 1.

A suitable simulation environment is an important step in screening different markets' decarbonization potential against most viable commercial steps and integration levels. Figures 2 and 3 below are the output of this tool and propose ROIs based on input on dryer sizing, -operating conditions, -temperature setpoints as well residual energy sinks and – not least - local utility costs.

ADVANCES IN EARLY WEANING IN FLATFISH: APPLICATION OF FLUID BED AGGLOMERATION FOR MICRODIET PRODUCTION

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Introduction

Early weaning in flatfish species remains one of the main bottlenecks in larval production, due to the high dependence on live feed and the limited efficiency of conventional microdiets. During early developmental stages, larvae possess an immature digestive system, which constrains the digestion of complex ingredients and limits the transition to inert diets. Therefore, the formulation of highly digestible microdiets tailored to larval physiological requirements is critical to improve weaning success (Hamre et al., 2013; Engrola et al., 2018). Extrusion technologies are widely used in commercial microdiet production due to their robustness and scalability. However, feed for early larval stages requires precise control of physical properties such as particle size, water stability, and nutrient leaching, as well as the ability to incorporate sensitive functional ingredients. Fluid bed agglomeration emerges as a promising alternative, enabling enhanced control over particle structure and functionality. This technology facilitates the inclusion of sensitive compounds such as protein hydrolysates, bioactive ingredients, and thermolabile nutrients while preserving their integrity and bioavailability. Protein hydrolysates have been shown to improve digestibility, feed acceptance, and digestive development in marine fish larvae (Kolkovski, 2001; Poonnual et al., 2025). Additionally, fluid bed technology allows optimization of key physical properties such as particle cohesion, water stability, and reduced nutrient leaching, which are essential for maximizing effective ingestion during first feeding and weaning stages. These characteristics position fluid bed agglomeration as a highly suitable technology for the development of species- and stage-specific microdiets. The aim of this study was to evaluate the potential of fluid bed agglomerated microdiets incorporating different protein hydrolysates, compared to commercial extruded diets, using two key aquaculture species: Senegalese sole (*Solea senegalensis*) and turbot (*Scophthalmus maximus*).

Materials and Methods

Two independent trials were conducted under controlled experimental conditions. In the Senegalese sole trial, larvae were obtained from natural spawning and the experiment lasted 21 days. Treatments were performed in triplicate: (1) commercial micro-extruded diet (control); (2) diet D1 produced by fluid bed agglomeration, including low molecular weight hydrolysate “A”; (3) diet D2 produced using the same technology, including higher molecular weight hydrolysate “B”; (4) diet D3 including a hydrolysate with a broad molecular weight range. Fish fed diets D1, D2, and D3 started co-feeding three days earlier than those fed the commercial diet. Dry weight, total length, and weight gain were evaluated. In the turbot trial, larvae were fed from mouth opening for 26 days with different microdiets: one commercial reference diet and two experimental diets (X1 and X2) with different protein hydrolysate profiles. Growth parameters (weight and length), relative growth rate (RGR), survival, feed intake, and feed conversion ratio (FCR) were recorded. In both trials, data were statistically analyzed after testing normality and homogeneity of variances, using parametric or non-parametric tests as appropriate ($\alpha = 0.05$).

Results and Discussion

In Senegalese sole, improved growth was observed with fluid bed agglomerated diets, with lower molecular weight hydrolysates further enhancing performance. In turbot, key parameters such as weight, RGR, survival, intake, and FCR did not differ significantly among treatments at the end of the pelagic phase. However, improved growth was observed during the first 10 days in larvae fed fluid bed agglomerated diets (X1 and X2). Previous studies have shown that protein hydrolysates can enhance palatability and digestibility in marine fish larvae (Kolkovski, 2001; Gisbert et al., 2012). Overall, results from both trials demonstrate that fluid bed agglomeration enables the development of microdiets with performance comparable or superior to conventional commercial diets. This is particularly relevant for larval nutrition, where ingredient composition and physical properties are critical for feed intake and utilization. The ability to design microdiets tailored to specific species and developmental stages positions this technology as a promising tool to improve early weaning efficiency in flatfish aquaculture.

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UNTANGLING BIOFOULING RISK: A CAUSAL FRAMEWORK APPROACH TO SEAWEED AQUACULTURE

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Background

Kelp aquaculture is rapidly expanding globally, mainly because of the diverse applications within food production, cosmetics and pharmaceuticals (Duarte et al., 2022; Kim et al., 2017; Liboureau et al., 2025; Pajot et al., 2022; Rosic, 2019; Stévant et al., 2017; Veenhof et al., 2024; Znad et al., 2022). However, in the nutrient-rich waters of the Northeast Atlantic, upscaling has been limited by complex interactions between abiotic and biotic factors (Førde et al., 2016; Delannoy et al., 2025). The larval or spore forms of organisms such as Bryozoans, Hydrozoans and macroalgae settle on the farmed seaweed fronds and cause biofouling (Bannister et al., 2019). Biofouling leads to substantial direct and indirect negative effects on the kelp, causing physical damage, halting growth, increasing the risk of consumer allergies and ultimately rendering the kelp unmarketable (Bannister et al., 2019; Martínez et al., 2024). For a sustainable Northeast Atlantic kelp farming industry it is thus key to understand the conditions that can predict biofouling timing and inform targeted interventions such as harvest time and optimal farm locations (Førde et al., 2016; Delannoy et al., 2025). Standard machine learning or statistical approaches are not sufficient to model the complexity of this system, which is characterised by complex interactions, time lags, confounding factors and non-linearity (Palaikostas, 2021; Utku, et al., 2025). In this study, we applied a causal inference framework, leveraging field data, to enable a mechanistic exploration of biofouling drivers as well as predictions under anticipated scenarios of environmental change.

Approach and preliminary findings

Samples were collected from ten kelp farms located across the United Kingdom, Norway, and Sweden from December 2024 to current day. At each farm, five sampling sites were established, where kelp blade, water, plankton, and environmental data were collected. Water and plankton samples were analysed at the Laboratory of Marine Diagnostics and Biotechnology, University of Glasgow, while kelp samples were processed for growth and biofouling metrics at the Marine Biological Association (MBA), Plymouth. Water samples were analysed for key water-quality parameters, including salinity, phosphate, nitrate, nitrite, and chlorophyll-*a* concentrations. Plankton samples were collected via net tows and examined microscopically to quantify phytoplankton species abundances (cells L⁻¹) and zooplankton class abundances (individuals m⁻³). Environmental and climatic variables, including sea surface temperature (SST), light availability, and current velocity, were obtained from Copernicus Marine Service and Open-Meteo datasets and calibrated using in situ logger measurements.

Descriptive analysis revealed substantial variation of environmental conditions across farms, particularly in nutrient levels, as well as seasonal shift among plankton communities. To investigate true environmental drivers of biofouling, a directed acyclic graph (DAG) was developed to formalise hypothesised causal relationships between environmental conditions, biological communities, and biofouling intensity. Gradient boosting machine (GBM) models were subsequently applied to evaluate predictor importance, identify optimal temporal lags, reduce model complexity, and inform refinement of the causal model.

(Continued on next page)

At the first stage of our analysis, we focussed on developing a taxon-specific causal model for bryozoan biofouling. Parameter estimates derived from this model were then used as informative priors within a broader whole-system framework encompassing six major epibiont groups: Amphipoda, Ascidia, Bivalvia, Bryozoa, Hydrozoa, and epiphytic macroalgae. This hierarchical approach facilitates the integration of taxon-level processes into a comprehensive model of biofouling dynamics in seaweed aquaculture.

The ultimate objective is to incorporate the causal framework into an interactive decision-support platform for seaweed farmers. The platform will allow users to input site-specific environmental conditions and management interventions and receive evidence-based, causally informed recommendations to optimise farm productivity and mitigate the impacts of biofouling.

Acknowledgements

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NOTES ON A NEOTROPICAL MIGRATORY FISH: SÁBALO

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Introduction

Native migratory fish species exhibit high mortality rates in the early stages of embryonic and larval development, making it important to study the early ontogeny of fish species of commercial and scientific interest (Adakebaike *et al.*, 2025). One of these native species of great economic and ecological importance in Brazil is the curimba (*Prochilodus lineatus*). It is a migratory species whose populations are threatened due to habitat deterioration, dam construction, and uncontrolled extraction. Furthermore, because it needs the stimulus of migratory environmental factors, its reproduction in captivity is problematic and must be artificially induced. This review aims to analyze the effect of certain factors, such as photoperiod, on the embryonic and larval development of *P. lineatus* in captivity, as well as the characteristics of water on sperm activation, taking into account the concept of phenotypic plasticity, with the goal of improving survival in both stages of development of the species.

Migration environment, sperm, larvae, and adaptation

Among the most important factors in migration and reproductive processes are photoperiod and gamete quality. In the latter, sperm motility is one of the fundamental aspects to study in order to improve fertilization and the production of quality embryos and larvae. In this sense, some studies have been carried out on the early stages of curimba ontogeny under natural photoperiod conditions, on the fertilizing capacity of semen and sperm morphology and on some ecological aspects. The larval stage has received less attention and the effects of changes in photoperiod on its development and growth are poorly understood. The compiled data indicate that there is growing interest in the study of gametes in native spawning species in Brazil and South America, particularly in the methodologies of cooling, freezing, and sperm quality assessment. In the case of curimba, these initiatives emerged mainly in the 1990s, with a progressive increase in the last decade.

Reports on the duration of sperm motility in this species have been similar to those reported for most teleosts, ranging from a few seconds to approximately three minutes. A prolongation of sperm motility in curimba or any other species would aid in the study of sperm physiology and could contribute to the creation of low-cost technological packages for the production of quality embryos, larvae, and fry. Similarly, the use of changes in light:darkness regimes has raised great expectations, as it has been proven to accelerate or delay reproduction and embryonic and larval growth and development rates in many fish species (Cowan *et al.*, 2024). However, there is a lack of information on these aspects in many Brazilian species of piracema, including *P. lineatus*. Thus, there is a need for research in which certain environmental factors, such as sperm activation solutions and photoperiod, are manipulated to improve curimba production in the early stages of its life cycle. Due to the nature of this research, the studies should be approached from the perspective of phenotypic adaptation or phenotypic plasticity of the biological model studied.

Acknowledgment

This review originates from a project on the reproduction of native fish in Brazil (Trad.). Thanks to the Federal University of Lavras, Brazil.

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SOME ASPECTS OF THE ECOPHYSIOLOGY OF REPRODUCTION IN CURIMBA (*Prochilodus lineatus*)

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This review aims to understand the effects of different photoperiods on the embryonic and larval development of curimba (*Prochilodus lineatus*) in captivity, as well as the implications of the composition of activation solutions on sperm motility, taking into account the concept of phenotypic plasticity, with the goal of improving production in this ontogenetic phase of the species.

Phenotypic Plasticity

Phenotypic plasticity (PP) is considered a general phenomenon where individuals modify their phenotypes in response to environmental signals, usually to adapt to changing environments. The reproductive strategies of teleost fish are very diverse, a phenomenon not evident in other vertebrates (Bose et al., 2025), but in most cases, eggs and sperm are developed separately by females and males in different modes and conditions of reproduction. Thus, migratory fish need the stimulus of river dynamics for the development of their reproductive system and gametes, and therefore, in environments with distinct characteristics, their reproduction is not possible or presents some difficulties that must be overcome. Among the migratory fish of Brazil is *P. lineatus*, a species distributed in the Paraná-Paraguay and Paraíba river basins, and of great economic and ecological importance among native medium and large-sized species. Its populations have been declining mainly due to commercial activities. There are also factors that can threaten fish populations, such as overfishing, habitat decline and alteration, and variations in water quality where these fish develop. Therefore, in vitro reproduction is of great importance in fish farming programs. Knowledge of the reproductive physiology of freshwater fish is still limited (Heins & Brown-Peterson, 2022), which is applicable to the circumstances of many neotropical countries. In conclusion, research is needed to determine the effect of activating solutions with different characteristics on fertilization and early ontogenetic development of *P. lineatus*. Likewise, it is important to study the development and growth patterns of the species under different environmental conditions, particularly photoperiod. The results that can be achieved in these future studies can be of great help in optimizing technological packages aimed at improving fertilization processes and the management of curimba embryos and larvae in captivity, taking into account PP.

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CAN MACROALGAE PROVIDE A CHEMICAL REFUGE FOR CALCIFYING SPECIES UNDER OCEAN ACIDIFICATION ?

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Introduction

The accumulation of CO₂ in the ocean causes acidification (Caldeira et Wickett 2003) and poses a significant challenge to calcifying organisms (Figuerola et al. 2021), threatening the sustainability of shellfish farming. One potential solution is to remove CO₂ by cultivating macroalgae. Through photosynthesis, macroalgae consume CO₂ to produce organic matter and oxygen in the presence of light. The co-cultivation of macroalgae and shellfish is already well established in other parts of the world, and several studies have emphasized the potential of macroalgae to mitigate the effects of acidification (Han et al. 2017; Wahl et al. 2018; Young et al. 2022; Gobler et al. 2026). However, the development of this practice in Europe using local species remains insufficiently explored. We therefore conducted complementary laboratory co-culture experiments to evaluate how this nature-based solution can mitigate the harmful effects of acidification on the Pacific oyster (*Magallana gigas*).

Materials and Methods

First, we defined the amount of macroalgae biomass required to offset the effects of acidification on oyster growth. We then compared the ability of three species of macroalgae to mitigate these effects: the red macroalgae *Palmaria palmata*, the brown macroalgae *Saccharina latissima* and the green macroalgae *Ulva sp.*, which occur naturally in Brittany and are of interest for aquaculture. Finally, we evaluated the effect of *P. palmata* on oyster growth and resistance to ostreid herpesvirus type I (OsHV-1) exposure. All tests were conducted in an open flow setup under conditions similar to those in the natural environment.

Results

Our results show that oyster growth is optimized under acidified conditions when the biomass of macroalgae is twice that of shellfish. Oyster growth under acidified conditions is higher in presence of *P. palmata* and *S. latissima* than with *Ulva sp.* or without macroalgae. Under ambient conditions, presence of macroalgae can reduce oyster growth, possibly due to significant diurnal variations in pH and dissolved oxygen levels. Finally, the presence of algae has no influence on survival of oysters exposed to OsHV-1, regardless of the acidification conditions.

Discussion

This study provides new insights into the mitigation potential of European species of macroalgae co-cultivated with oysters, consistent with previous studies (Han et al. 2020; Wahl et al. 2018; Young et al. 2022; Young et Gobler 2018) oyster and seaweed. The effect of macroalgae on oysters may depend on various factors, including the biomass ratio and the species selected. The 2:1 ratio should be considered optimal within the constraints of our experimental setup. In small tanks, higher macroalgal biomass may induce self-shading and reduce photosynthetic performance. The species-specific effects of macroalgae on seawater parameters, such as pH and dissolved oxygen, may have contrasting impacts on oyster growth. Daily pH fluctuations driven by photosynthesis can influence calcification and growth depending on the photoperiod in *Mytilus edulis* (Wahl et al. 2018). These daily oscillations may explain why oysters grew better without macroalgae under ambient conditions but not under acidified conditions. Dugeny et al. (2022) demonstrated that macroalgae influenced oyster microbiota composition, which in turn, affected survival after OsHV-1 exposure. Although there was no significant effect of macroalgae on survival after OsHV-1 exposure, the potential impact on the microbiota will be investigated in future analyses. Our results suggest that macroalgae can provide a chemical refuge for calcifying species under ocean acidification.

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COMPARISON OF THREE METHODS FOR NEPHROCALCINOSIS DIAGNOSTICS IN FARMED RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Nephrocalcinosis in Rainbow trout is a well-known problem in Norwegian fish farming¹ and considered the most important problems in the freshwater stage². With increasing production and use of new production systems, the need for new, non-invasive or non-lethal diagnostic methods such as radiography³ and ultrasound is emerging, to give consistent evaluations and diagnostics. Now a variety of scoring systems and methods are used for nephrocalcinosis assessment, and their respective strengths and limitations are not fully understood.

Material and methods

A total of 777 Rainbow trout were examined using radiography and macro scoring to assess nephrocalcinosis severity. A subset of 24 fish were selected for histological examination, widely regarded as the reference method for assessing renal pathology in nephrocalcinosis. The three methods evaluated were: radiography (detection of mineral deposits throughout the kidney), macro scoring (gross pathological changes on the kidney surface), and histology (cellular-level characterisation of renal pathology).

Results and discussion

Agreement between methods was highest in moderate to severe cases of nephrocalcinosis. In low-grade cases, histology revealed structural changes that were not consistently detected by radiography or macro scoring. Notably, some of the histologically most severe cases showed low radiographic and macro scores. These kidneys were characterised macroscopically by fibrosis and altered texture and colour, and histologically by extensive tissue damage, including loss of interstitial tissue with key roles in haematopoiesis, immune function and hormone production.

These findings indicate that the different diagnostic methods capture distinct aspects of nephrocalcinosis, with radiography reflecting mineral burden, macro scoring reflecting superficial structures and histology revealing structural tissue damage. The results further suggest that current macro scoring systems may benefit from including additional descriptors such as texture, contour and colour in order to better reflect chronic pathological changes.

Acknowledgment

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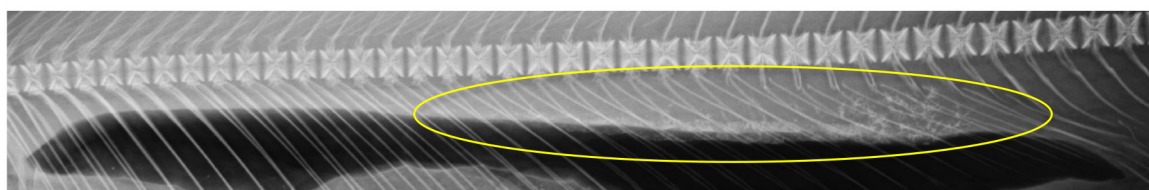


Figure 1: Radiograph of Rainbow trout kidney with nephrocalcinosis score 3.

THE PARADOX OF NUTRIENT EMISSIONS IN NORWEGIAN FJORDS: THE PROBLEM IS TOMORROW, NOT TODAY

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Main message

- Aquaculture is the largest anthropogenic source of nitrogen and phosphorus to the Hardangerfjord.
- Monitoring data show good to very good environmental status for nutrients and chlorophyll.
- Model simulations show that increased aquaculture production can significantly increase primary production in some fjord areas.
- Absence of observed effects does not mean that the system has the capacity for further growth.

Background

In the Hardangerfjord at the western coast of Norway, annual aquaculture production is over 100,000 tons of salmon and is the largest anthropogenic source for excess nutrients. Environmental impacts of aquaculture are a major public concern in Norway, despite monitoring tells a different story: in 2024, seven of eight dissolved nutrients monitoring stations and all seven chlorophyll-*a* monitoring stations were classified as having very good ecological status (Figure 1). A management question arises: does good ecological status indicate remaining environmental capacity, or does it merely describe current conditions?

Results and discussion

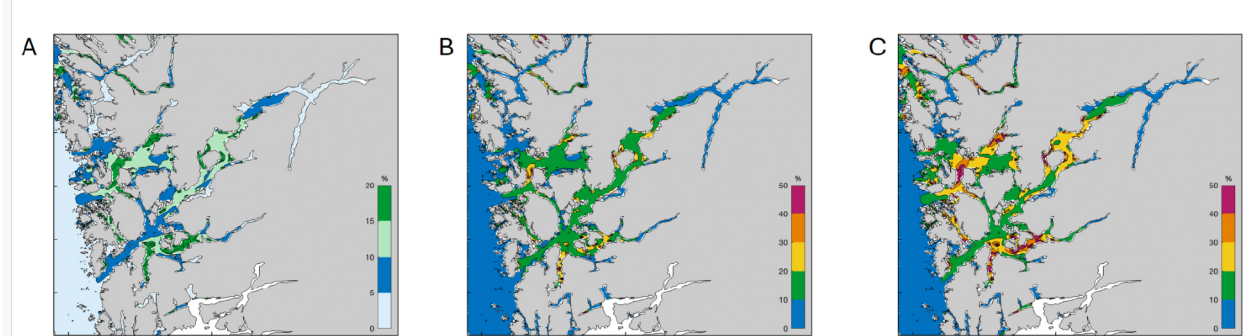
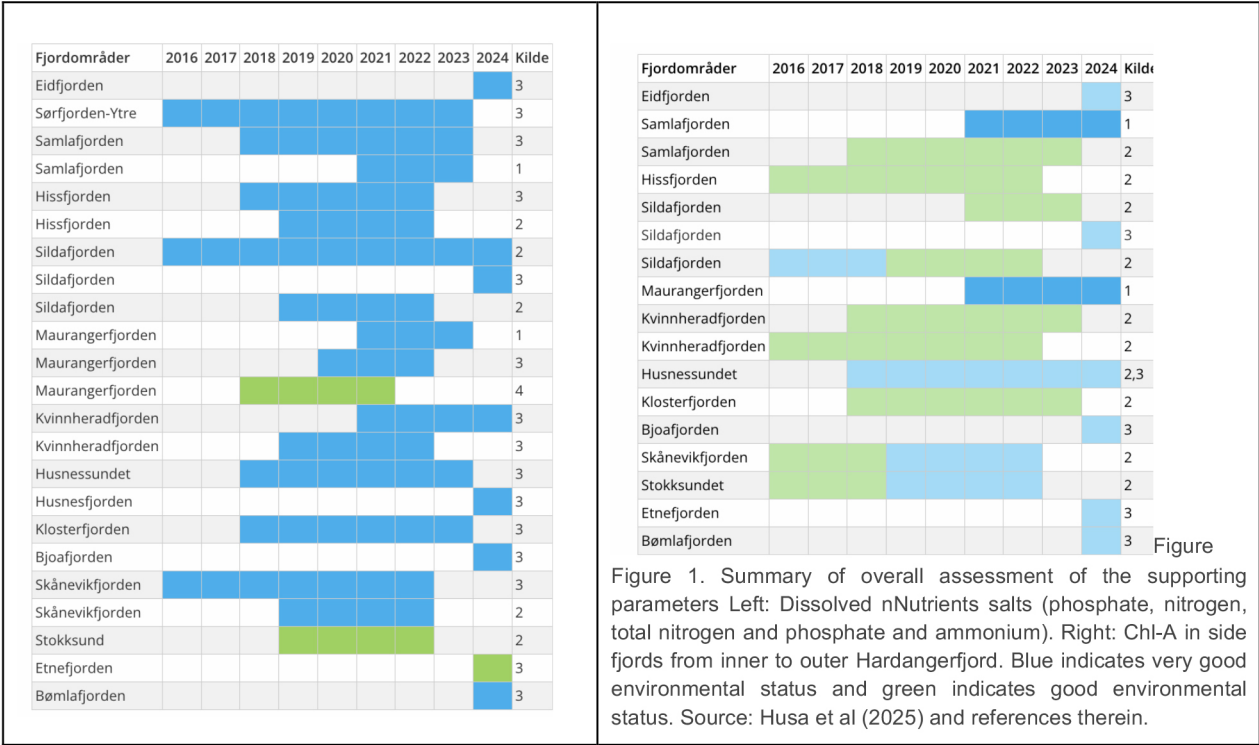
Remaining spare capacity for aquaculture production in the Hardangerfjord was assessed. Spatial varying annual primary production responses due to excess nutrients release were calculated with the ecosystem model NORWECOM.E2E (Hjøllo et al 2025). We compared a reference simulation without excess nutrients from aquaculture to 3 simulations using i) current aquaculture production (Figure 2 A), ii) current + planned increase in production (Figure 2B) and iii) doubled production. Corresponding nutrients were released at sites reporting production in 2024. Modelled responses (Figure 2A) show good ecological status today, with no widespread nutrient enrichment observed, although spatial hotspots emerge. Increased phytoplankton production under scenarios ii and iii indicated increasing risks for eutrophication in both the main fjord and the sidefjords.

In summary, the Hardangerfjord monitoring says: “Everything looks fine”, while modelling says: “Not for much longer”. Environmental monitoring is designed to detect impacts, not to quantify remaining carrying capacity. Recently, the management authority for aquaculture production (County Governor of Vestland) challenged indicator-based regulation of aquaculture nutrient emissions. All applications for further production increase in the Hardangerfjord were turned down based on negative trends in some monitored environmental parameters (e.g., macroalgae and oxygen) (Pedersen et al 2025), which together with the expected continuation of climate change impacts, is likely to reduce the Hardangerfjord’s capacity to receive and assimilate additional emissions. The County Governor therefore concluded that a rise in aquaculture production is not sustainable. In many areas of environmental management, including climate adaptation and natural hazard management, decisions are routinely based on projected future risk rather than waiting for observable ecological deterioration. Should aquaculture management take the same pre-cautionary approach?

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PHOSPHONATE CYCLING BY MARINE PHYTOPLANKTON

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Introduction

Phosphonates, compounds with carbon-phosphorus bonds, are common in the marine environment and can be used as source of cellular phosphorus (P) in bacteria through the action of several hydrolytic enzymes (PhnA (McMullan & Quinn, 1992), PhnX, PhnW, PhnY, PhnZa (McSorley et al., 2012), and PhnZc (Gama et al., 2016). The recent discovery of homologous genes in eukaryotes has raised questions about whether photosynthetic and heterotrophic protists can obtain P using similar enzymes, particularly in P-limited environments. This study investigates the functional expression and regulation of these genes under phosphorus limitation to better understand their potential roles in nutrient stress adaptation and their impact on marine primary productivity.

Material and Methods

Saccharomyces cerevisiae was used as a heterologous expression system to evaluate the functionality of phosphonate-degrading enzymes from *Perkinsus marinus* and *Effrenium voratum*. The *phnA* and *phnYc-phnZc* genes were expressed from episomally replicating plasmids or integrated at defined genomic loci via homologous recombination. Genes of interest were fused to GFP or Myc tags for localisation and expression analyses. Growth assays using phosphonoacetate and methylphosphonate as sole phosphorus sources were monitored by optical density (OD₆₀₀) measurements. Constructs lacking the mitochondrial targeting presequence were generated to assess mitochondrial localisation. Phosphonate utilisation was further evaluated in native phytoplankton species harbouring *phn* pathways using flow cytometry-based growth measurements. *E. voratum* harbouring *phnYc-phnZc* and *Gephyrocapsa huxleyi* harbouring *phnZY* pathways were grown with methylphosphonate and 2-aminoethylphosphonate (2-AEP) as phosphorus sources, with flow cytometry data analysed using FlowJo.

Results

Yeast strains expressing episomal *phnA* and *phnYc-phnZc* constructs exhibited restored growth on phosphonoacetate and methylphosphonate, demonstrating the ability to utilize phosphonates as a phosphorus source. This phenotype was absent in strains lacking the mitochondrial targeting presequence, indicating that mitochondrial localization is required for pathway functionality. GFP-tagged proteins showed colocalisation with MitoTracker staining, confirming mitochondrial localisation of *phnA*, *phnYc-phnZc*, and additional pathway-associated proteins (PhnX, PhnW, PhnY, and PhnZa). Flow cytometry-based growth assays in *E. voratum* harbouring the *phnYc-phnZc* pathway and *G. huxleyi* harbouring the *phnZY* pathway further confirmed phosphonate utilisation in marine phytoplankton. Growth was supported using methylphosphonate and 2-AEP as phosphorus sources, demonstrating that these pathways enable phosphonate degradation and contribute to phosphorus acquisition beyond the heterologous yeast system.

Discussion

This study provides novel insights into the functional capacity of marine protists to access alternative phosphorus sources through phosphonate metabolism. The restoration of growth in yeast expressing *P. marinus* and *E. voratum* phosphonate-degrading enzymes demonstrates that these pathways are functional and highlights the importance of correct subcellular targeting for phosphonate utilisation. The requirement for mitochondrial localisation suggests that phosphonate degradation involves specialised intracellular processes that enable access to alternative phosphorus sources under nutrient-limited conditions. The identification of functional phosphonate metabolism in both a parasitic protist (*P. marinus*) and phytoplankton species (*E. voratum*, *G. huxleyi*) expands our understanding of the diversity of organisms capable of utilizing phosphonates. Furthermore, validation of phosphonate-supported growth in native phytoplankton demonstrates that these pathways can contribute to phosphorus acquisition beyond a heterologous expression system. These findings reveal a previously unrecognised strategy among diverse marine protists for exploiting alternative phosphorus sources, with potential implications for nutrient availability and microbial interactions in marine ecosystems. Understanding the distribution and function of these pathways provides new perspectives on how aquatic microorganisms respond to changing nutrient regimes and highlights the importance of incorporating microbial eukaryotes into models of marine phosphorus cycling and ecosystem resilience.

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PREDICTING MUSSEL GROWTH USING ENVIRONMENTAL VARIABLES AND TWO MODEL APPROACHES

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Introduction and methods

Aquaculture can contribute to the worldwide demand for sustainably produced protein. With a yearly production of around 400 million kg in the European Union, the blue mussel (*Mytilus edulis*) is commercially important. New production areas need to be sought to upscale production and current areas might be used more efficiently. Computer models relating mussel growth to environmental variables such as food availability and temperature help understand the spatial and temporal variation in observed mussel growth.

Large scale, high resolution environmental data (chlorophyll-*a* concentration as proxy for food quantity, total suspended matter as modifier for food quality, irradiance, and temperature, all measured near the sea surface) were obtained from remote sensing (Sentinel-3 satellites). Models were trained and validated with a set of standardized measurements of monthly weight increment (grams total individual wet weight) of mussels on 12 mussel aquaculture plots in the Eastern Scheldt area, The Netherlands. These measurements were repeated yearly from 2018 to 2022.

Two model approaches were applied: (1) a data-driven (DD) model to find correlations between environment and measured mussel growth, unconstrained by forced relationships, and (2) a mechanistic Dynamic Energy Budget (DEB) model based on theoretical relationships between environment and physiology. Both models generated predicted weight-over-time series for a growing season (April – November) for the 12 locations in the Eastern Scheldt for several years. The models were trained and validated with leave-one-out cross validation whereby the location-year combination for validation is always different from the locations and years used for model training.

Results and Discussion

Comparing a data-driven (DD) model with a Dynamic Energy Budget (DEB) model yielded valuable insights in how blue mussels (*Mytilus edulis*) respond to their environment. The DD model was severely hindered by seasonal confounding of explanatory variables. Seasonal patterns in irradiance, sea-surface temperature and growth of the mussels were highly consistent across locations and all coincided with seasonal variation in growth. Consequently, the DD model was not able to distinguish between their effects. Contributions of chlorophyll-*a* concentration and total suspended matter were not statistically significant for the DD model, even though some unexpected correlations were revealed in the data. DD model predictions for the validation data were not significantly correlated to observed growth.

The DEB model was able to produce a small, but significant positive correlation between predicted growth and observed growth. This correlation was mainly due to variation in chlorophyll-*a* concentrations as spatial variation in temperature in the Eastern Scheldt is limited. Some of the spatial and temporal variation in observed mussel growth might be explained better after inclusion of another proxy for food quantity than chlorophyll-*a* (e.g. primary production), or a more appropriate proxy for food quality (e.g. species composition). Extrapolating temperature effects to novel locations with, for example, lower temperatures, may allow scenario testing for translocating mussels to promising new aquaculture locations.

ADVANCING COMPETENCE IN RECIRCULATING AQUACULTURE SYSTEMS FOR THE NORWEGIAN ATLANTIC SALMON INDUSTRY

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Introduction

The rapid expansion of Recirculating Aquaculture Systems (RAS) in the Norwegian Atlantic salmon (*Salmo salar*) industry has created a critical demand for specialized technical and biological expertise. To address this “competence gap,” the life-long learning course in RAS biology was established as a collaborative effort between academia, research and industry partners to provide advanced training for professionals already working within the sector.

Methodology

The course is structured around three intensive physical gatherings focusing on four main topics: the first gathering on RAS technology, the second on microbiology and water quality, and the third on fish health and welfare. The pedagogical approach combines lectures from international and national experts with hands-on laboratory work, including welfare scoring (Nilsson et al., 2025), and microbial and water chemistry analysis. Lab work is reported in the form of a lab journal. Each gathering includes visit to a commercial RAS facility. Relevant “case studies” from everyday operation of commercial RAS are discussed in groups and in plenum. Between the physical meetings, digital seminars with equipment suppliers, salmon producers, researchers and feed manufacturers are organized to ensure a holistic understanding of the RAS value chain. After each gathering the participants are given assignments related to the topic discussed at the gathering, which is presented orally and is a part of the final exam. The course is given once per year, provides 15 study points and is limited to maximum 15 participants.

Results and Discussion

Since 2023, the program has successfully arranged three courses and trained over 30 participants from salmon producers, including Mowi, SalMar, Lerøy Aurora, Grieg Seafood, Arnoy Laks, Astafjord Smolt and Elvevoll Settefisk. In addition, staff from Tromsø Aquaculture Research Station and lecturers from the Vocational college in the North participated in given courses. This course provided a platform for networking that allows for the transfer of best practices across companies, reducing the risk of common operational pitfalls. Participants received state-of-the-art knowledge about salmon production in RAS and were able to see chosen technical and management solutions at other commercial sites. Preliminary results suggest that this targeted continuing education model significantly enhances the operational reliability of commercial RAS facilities in Norway.

Course evaluations and oral exam presentations demonstrate a clear increase in participants’ understanding of key RAS challenges, including system–biology interactions, microbial management, water quality control, and welfare indicators in intensive production. Participants reported increased confidence in interpreting water chemistry and microbiological data, applying welfare scoring tools, and critically assessing technical and operational solutions in their own facilities. Exposure to multiple commercial RAS sites and contrasting management strategies enabled participants to benchmark practices and reflect on how design and operational choices influence fish health and biological performance.

The course also functioned as a professional networking arena, facilitating the transfer of best practices across companies and regions. Group-based case work based on real operational challenges stimulated learning and highlighted common risk factors in RAS operation, such as biofilter instability and system start-up phases.

Overall, these results suggest that a structured, practice-oriented continuing education model can contribute to strengthening operational robustness and biological competence in commercial RAS facilities. The course framework provides a scalable template for future life-long learning initiatives aimed at supporting sustainable growth of land-based salmon production in Norway.

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Acknowledgment

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QUANTIFICATION OF FISH FEEDING ACTIVITY BASED ON TAIL BEAT FREQUENCY MEASURED USING AN OPTICAL CAMERA

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Introduction

In Japan's aquaculture industry, feed amounts, feeding times, and feeding frequencies are set daily by farmers' experience and judgment based on the season and rearing conditions. These parameters are almost always determined personally based on visual observations and the experience of the workers. Therefore, a key challenge is to establish a technology that can objectively and quantitatively evaluate fish activity automatically and contribute to the determination of feeding parameters. While there are numerous technologies for measuring fish activity based on behavioral parameters (Kawabe et al., 2003; Sasaki et al., 2024), given practical constraints, the simpler and more robust the method, the better. Therefore, in this study, we focus on a tail beat frequency, which correlates with the fish's swimming speed (Bainbridge, 1958), and propose a method for non-invasively measuring the tail beat frequency using a single optical camera. We applied image processing and machine learning techniques to video obtained from a single optical camera to extract feature points on the fish's body and automatically measure the tail beat frequency based on the time-series changes in their positions.

Materials and Methods

In a circular submersible fish cage (30 m in diameter) located in the southern part of the Japanese archipelago, where yellowtail (*Seriola quinqueradiata*) are farmed, a camera was installed in the surface layer of the fish cage, oriented toward the bottom of the net. Using this camera, we recorded the swimming behavior of yellowtail during and around feeding times. We continuously performed object detection using YOLOv8 (Jocher et al., 2023) and video segmentation tracking using SAM2 (Ravi et al., 2025) on the acquired video to obtain time-series data on the coordinates of the yellowtail's caudal fin within the frame. We also manually tracked the caudal fins of the yellowtail in the footage and compared the results with those from the AI-based automatic measurements.

Results

Figure 1 shows a representative example of AI-based automated tracking. In this monitoring experiment, approximately one hour of video footage, including the feeding period, was recorded. Both manual and automated measurements showed that tail beat frequency increased at the onset of feeding and then gradually decreased toward the end of the feeding period.

Discussion

Although the results of manual and automated measurements generally showed similar trends, while AI-based automated measurement could process a very large number of samples, errors at each stage of the analysis tended to accumulate, leading to greater variability in the output values. Furthermore, it was confirmed that the swimming behavior observed varied depending on the camera's position within the fish cage. It is thought that the closer the camera is to the center of the cage—near the area where feed is dropped—the higher the observed activity level.

This study demonstrated that AI-based automatic analysis can capture the trend of increased the tail beat frequency during feeding. Taking into account the areas for improvement identified in this experiment regarding analytical methods and operational implementation in real environments, we plan to address these improvements to enhance the calculation speed and reduce errors in AI-based automatic measurements.

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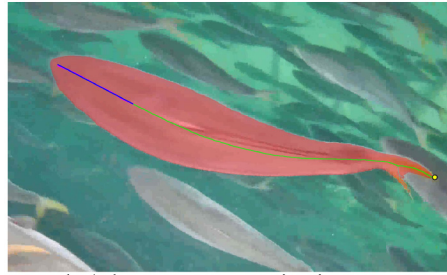


Figure 1

The red area represents the mask assigned to the fish, the green line indicates the centerline of the mask, the blue line indicates the body axis of the fish, and the yellow point represents the coordinate of the tip of the caudal fin.

Acknowledgment

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AUTOGENOUS OSHV-1 VACCINATION OF PACIFIC OYSTER: SURVIVAL BENEFITS AND TRANSCRIPTOMIC MECHANISMS OF PROTECTION

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Pacific oyster mortality syndrome (POMS), caused by Ostreid herpesvirus-1 (OsHV-1), represents one of the most significant threats to global oyster aquaculture. Our research group has recently demonstrated that oysters are amenable to immune priming, supporting the potential for vaccination-based approaches. Autogenous vaccination therefore offers a promising strategy to confer protection, yet the host immune mechanisms underlying vaccine-induced protection remain poorly understood. Here, we evaluated the protective efficacy and host transcriptional basis of immune priming in *Crassostrea gigas* using heat-treated and live OsHV-1 vaccines across two temperature conditions, combining survival analysis with whole-transcriptome profiling.

All vaccinated groups showed significantly improved survival following OsHV-1 challenge compared to unvaccinated controls, with relative percent survival values of 76.2% and 85% for a heat-treated vaccine delivered at 22°C and a live OsHV-1 vaccine delivered at 18°C. Vaccinated oysters also showed markedly reduced viral loads and lower OsHV-1 viral transcript abundance following challenge compared to unvaccinated controls, with the live OsHV-1 priming group exhibiting significantly lower viral DNA concentrations in moribund oysters ($p = 0.001$), collectively indicating effective suppression of viral replication in primed animals. Transcriptome profiling across 51 samples revealed temporally dynamic and treatment-specific transcriptional responses. During priming, the live vaccine group mounted an earlier response (1,167 differentially expressed genes, DEGs, at 1 dpp vs. 537 in the heat-treated group), whereas the heat-treated group exhibited a substantially amplified peak response of 3,908 DEGs at 4 dpp. Following viral challenge, the unvaccinated positive control showed the largest transcriptional response, peaking at 879 DEGs at 4 dpc and remaining elevated at 316 DEGs by 12 dpc, while both vaccinated groups resolved to 45 and 82 DEGs by the same timepoint.

These quantitative differences revealed that the two vaccine formulations engaged distinct molecular entry points into the host immune response. Live OsHV1 rapidly activated RLR/MAVSlike antiviral pathways and interferonstimulatedlike transcriptional responses within 1 day postpriming, consistent with direct recognition of viral nucleic acid, whereas the heat-treated group triggered a delayed but markedly amplified remodelling of proteostasis networks, ubiquitin–proteasome machinery and intracellular trafficking at 4 days postpriming. Upon subsequent viral challenge, the live vaccine group showed strong early upregulation of DNA replication and cell cycle modules, including genes most similar by orthology inference to replication licensing factors MCM2, MCM4 and MCM7, and DNA polymerase epsilon—consistent with expansion of the haemocyte compartment—alongside IFI44Lfamily and DHX58/LGP2like antiviral response genes. In contrast, unvaccinated oysters sustained a prolonged interferonstimulatedlike response characterised by upregulation of interferonstimulatedlike genes and dsRNA sensorfamily genes, alongside BIRC2like inhibitor of apoptosis genes, persisting through 12 days postchallenge without resolution. These findings demonstrate that autogenous OsHV-1 vaccination reconfigures rather than suppresses the host transcriptional response to viral challenge, providing a functional transcriptional basis for vaccine-induced resilience against POMS and supporting the development of autogenous vaccination as a viable biocontrol strategy for oyster aquaculture.

Acknowledgment

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AMINO ACIDS SUPPLY-DEMAND PITFALLS IN EUROPEAN CARP PONDS AND ITS FULFILLMENT BY PRECISION POND FEED

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Introduction

In most European fishponds culturing cyprinids, natural food and supplemented cereals such as wheat is the business as usual. The protein and indispensable amino acid (IAA) quantity x quality of natural food webs fluctuate seasonally. The carp growth, N:P retention or excretion decisions are at the mercy of that, ranging from very good to very bad across the vegetative season. The supply-demand dynamics of some critically limiting IAAs such as lysine (LYS) and methionine (MET) remain poorly understood, as part of nutritional ecology of aquaculture ponds. It is not only the case of European fishponds, but also a globally relevant question. The present study had two objectives. *Pre-trial*: to develop a model of supply-demand of LYS and MET under traditionally grain-fed (<10% CP) or other low-protein pellet-fed ponds (<25% CP). *Main-trial*: to develop and test a precision-balanced pond feed (at the threshold of protein feed nomenclature, *i.e.*, 30% CP) that solves the supply-demand gap of LYS-MET in ponds through a carp nutritional bioenergetics assay and simultaneous validation in pond for proof-of-concept.

Materials and Methods

Pre-trial: Two groups of ponds were selected (Group-A, n=3 ponds: fed by wheat alone, 10% CP during April–October; Group-B, n=6 ponds: fed by wheat during April–June and low-protein plant-pellet 23% CP during July–October, either by hand or demand-feeder) at the Faculty of Fisheries and Protection of Waters research station, Vodnany stocked with stocked with 2+ age *Cyprinus carpio* (384.82±3.47 g; 471 kg ha⁻¹; 1183±23 individuals ha⁻¹). Monthly plankton consortia samples were collected from nine earthen experimental ponds (0.16 ha area, 0.8 m depth), using a 200 µm-mesh plankton net (mimicking branchial sieve minima of common carp >200 g), lyophilized, and stored at –20 °C. Samples were hydrolyzed using two procedures: acid hydrolysis (6 M HCl, 108 °C, 23 h) for total AAs, and oxidative hydrolysis (formic acid/H₂O_{ss} followed by 6 M HCl, 120 °C, 23 h) for cysteine and methionine. After evaporation and reconstitution, AAs were separated by ion-exchange chromatography on an AAA 500 analyzer with post-column ninhydrin derivatization and photometric detection at 570 nm and 440 nm.

Main trial: precision pond feed (31% CP) was formulated in WinFeed using 70% LOCB (low-opportunity-cost-biomass, comprising corn DDGS, rapeseed expeller meal, sunflower expeller meal, and <5% freshwater fishmeal from fish processing) and 30% human feed-food conflict ingredients (such as wholewheat meal and rapeseed oil). The IAA profile was carefully calibrated using crystalline L-lysine hydrochloride and DL-methionine (maintaining stoichiometry 3:1) in a way that the IAA profile mimics zooplankton-zoobenthos standards (in terms of DIAAS_{LYS,MET} >50%) and the feed fulfills ≥90% of LYS, MET requirements of carp. The starch (21%) and lipid (8%) were carefully balanced to fulfill ≥100% non-protein energy requirement of carp. It also has enough starch (>20%) for high-quality extrusion of pellets. A high non-starch polysaccharide (NSP 33%) contributed by LOCBs were accepted as filler but given that NDF:ADF ratio is >2 (for making the fibers easily fermentable in carp gut or by pond).

Precision pond feed was evaluated against wheat (as a control) for apparent digestibility, metabolic losses, and retention of protein in our Guelph-RAS system using standard methodologies. For this purpose, the lab trial lasted 70-day with common carp (initial body weight ~20g; 23–27 fish per tank) randomly assigned to 8 tanks across two treatments: pond feed and wheat with four replicates each (fed 6% BW day⁻¹, split into two doses at 08:00 and 14:00) and uneaten pellets dried, measured (40 mins post feeding). Following clearing of uneaten pellets after 2nd feeding, passive faeces collection continued every 4 hours until next day feeding. Initial and final carcass were taken for whole-body homogenates for protein, making sure that the duration of experiment has passed until one of the treatments had tripled body weight.

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Simultaneously, a pond validation trial with 2+ age carp (640–660 g) was conducted in 9 ponds (same as pre-trial) but challenged by a higher stocking density (625 kg ha⁻¹; 975 individuals ha⁻¹). Control ponds (n=3) received wheat during May–October. Same total dose treatment ponds (n=6) received as wholewheat or extruded breakfast cereals during April–June, and precision pond feed during July–October. Ponds were equipped with underwater temperature data loggers to precisely compute degree-days of culture, and fish measurements done monthly (n=20 pond⁻¹ month⁻¹) to compute inter-month thermal growth coefficient (TGC). Monthly monitoring of water quality (nutrients, chlorophyll-a) and zooplankton biomass (200–500 µm; >500 µm) was monitored.

Results

Pre-trial: During May–June, the supply of LYS (70–121 g) and MET (24–46 g) significantly exceeded ($p < 0.05$) pond carp's demand (LYS: 32–59 g; MET: 9–18 g). July acts like a transition period when supply and demand of LYS and MET are in equilibrium ($p > 0.05$). From August onwards, LYS supply fell to 34–54 g ($p < 0.05$) against a demand of 91–115 g, and MET supply dropped to 15–24 g ($p < 0.05$) against a demand of 27–34 g. This late-season drop coincided with decline of zooplankton (and zoobenthos) in pond, but an increase in algae (chlorophyll-a). Overall, the pattern shifted from early-season IAA surplus to late-season IAA limitation. Selecting any low protein level with any random IAA profile such as the low-protein pellet fed in group-B ponds (23% protein, fulfilling 40% of carp's LYS requirement, 66% MET requirement) over wheat fed in group-A ponds (10% protein, fulfilling 12% LYS requirement, 23% MET requirement) does not work. Such imprecise interventions do not solve the late-season IAA deficit of pond carp, as the net yield and relative feeding coefficient (RFC) of group-A and group-B ponds were same ($p > 0.05$).

Main trial: Application of precision pond feed during the problematic period of IAA supply-demand (July–September) had solved the issue of depressed growth in carp, compared to control ponds. In control, the TGC of pond carp stock collapsed during July–September. In treatment, during the same period, the TGC of pond carp was significantly reinforced compared to control ($p < 0.05$), that too with consistently high TGC across the months July to September ($p > 0.05$). This led to significantly high net yield (1.7 tons ha⁻¹), bigger market-sized carp (2.4 kg, market-size 2 kg already in September), and RFC (2.5) in treatment ponds ($p < 0.05$) than the control ponds (1.2 tons ha⁻¹; weight 1.9 kg; RFC 3.4). The water quality was also controlled. Despite such high production and application pellets in treatment pond, the end-of-culture (August–November) concentrations of nutrients (C: N:P) in water or sediment, and chlorophyll-a concentrations were same as control ($p > 0.05$). The large-bodied zooplankton (>500 µm) biomass was significantly ($p < 0.05$) enhanced in treatment (5–24 mg L⁻¹) over control ponds (3–12 mg L⁻¹) during August–October, indicating natural food could regenerate when deficiency for fish was lifted. A high bioenergetic efficiency of precision pond feed over wheat was confirmed in the lab trial. Protein storage efficiency of precision pond feed (net-stored protein 40% of digested protein) was significantly greater ($p < 0.05$) than that of wheat (net-stored protein 30% of digested protein). Net-storage of amino acids from the digested pool *in-vivo* is presently under construction.

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BACTERIAL BIOCONTROL AND DIETARY PROBIOTIC INFLUENCE SAPROLEGNIOSIS RESISTANCE IN ATLANTIC SALMON

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Introduction

Saprolegniosis is a widespread freshwater fish disease caused by several species of the genus *Saprolegnia*, predominantly *Saprolegnia parasitica* and *S. diclina*. The disease affects all developmental stages, from egg to adult, and is most often opportunistic, developing in stressed, injured, or coinfecting individuals. Preventive health management is paramount in mitigating the risk of outbreaks and curative treatments. Microbial-based strategies, both in-feed or by immersion, have the potential to enhance fish health and robustness but remain underlooked in their capacity to mitigate *Saprolegnia* dissemination and the risk of outbreaks.

Material and Methods

In this study, two complementary approaches were used. First, three strains of *Saprolegnia* (*S. diclina*, *S. parasitica* and *S. ferax*) were tested *in vitro* to screen, amongst many, bacteria candidates best able to inhibit the growth of *Saprolegnia* oomycetes. To do so, bacteria and *Saprolegnia* strains were co-cultured on plate and on flask and the growth of the oomycete was measured after 1 week. Second, an *in vivo* trial was performed testing a yeast-based functional ingredient alone or in combination with one of three live-bacteria selected from *in vitro* screening and applied by immersion. To do so, freshwater Atlantic salmon (BW = 20 g) were fed to apparent satiation a control diet or the same diet supplemented with a yeast-based functional ingredient selected from prior knowledge. After 5 weeks of nutritional preparation, fish were randomly distributed into 24 tanks (12 tanks / diet group). Fish from each dietary origin were then exposed to one of four biocontrol treatments (Blank Control, Biocontrol A, B or C) applied in triplicate by 1h static immersion under aeration. Immediately after biocontrol exposure, fish were exposed to a controlled *S. parasitica* bath challenge and mortality was recorded daily during eighteen days. Skin and gill tissues were sampled at intervals for microbiota, histology and gene expression analyses.

Results

In-vitro screening allowed to select two *Bacillus velezensis* strains with a high growth inhibition capacity against three *Saprolegnia* strains. One of the *B. velezensis* strain was selected for *in vivo* testing in combination with *Pediococcus acidilactici* (Biocontrol C). Following *S. parasitica* challenge, mortality dynamics differed markedly between groups. When fed the control diet, mortality started earliest in the absence of biocontrol exposure and was clearly mitigated when exposed to Biocontrol C while Biocontrol B and A had intermediary and no benefits respectively. In contrast, in-feed supplementation with the yeast-derivatives slightly improved survival compared to the non-supplemented diet and overall reduced the apparent benefit of biocontrol exposure. Nonetheless under this regime, Biocontrol C followed by Biocontrol B reduced mortality hazard from day 8 post-challenge.

These outcomes reveal a strong diet-dependent interaction shaping host resilience and disease progression. Ongoing tissue analyses will be instrumental in elucidating the mechanisms underpinning the health benefits associated with Biocontrol C.

Conclusion

The trial demonstrates that both diet and biocontrol treatment significantly mitigate mortality following *Saprolegnia* exposure. Supplementation with the yeast-based ingredient provided the greatest single survival benefit, while Biocontrol C delivered the most consistent protective effect across dietary conditions by modulating the timing and intensity of mortality risk. Overall, the findings highlight the potential of microbial-based biocontrol strategies to mitigate saprolegniosis in freshwater salmonids. The consistent modulation of mortality dynamics by Biocontrol C, coupled with its dependence on dietary background, emphasizes the importance of integrating functional nutrition with targeted microbial interventions to enhance fish robustness.

EXPLORING FEED- AND BATH-BASED MICROBIAL STRATEGIES TO ENHANCE EUROPEAN SEABASS (*Dicentrarchus labrax*) RESILIENCE AGAINST *Aeromonas veronii*

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Introduction

Aeromonas veronii is a Gram-negative bacterium affecting European seabass (*Dicentrarchus labrax*) in the Mediterranean region since 2008 (Smyrli *et al.*, 2017). The bacterium causes a severe systemic disease characterized by external haemorrhagic lesions and internal pathological alterations, leading to significant economic losses due to mass mortalities especially of fish reaching commercial size. Outbreaks typically occur during the warmest months of the year, when water temperatures exceed 21°C, and cumulative mortality can surpass 50% in the absence of effective antibiotic treatment (Smyrli *et al.*, 2019). As concerns over antibiotic use continue to rise, preventive health management strategies are becoming increasingly important for mitigating disease risk. Microbial interventions, delivered either through feed or immersion, have shown potential to enhance fish health and resilience. However, their capacity to reduce disease severity and mortality following experimental *A. veronii* exposure remains largely unexplored.

Material and Methods

In this study, two complementary approaches were used. First, two strains of *A. veronii* were tested *in vitro* to screen a panel of candidate bacteria for their ability to inhibit pathogen growth. For this purpose, *A. veronii* strains were exposed to secondary metabolites of various bacteria, and optical density at 600 nm (OD600) was monitored over 48 h to assess pathogen growth. Second, two independent *in vivo* trials were conducted to evaluate microbial-based strategies for mitigating the impact of *A. veronii* in European seabass. Across the two trials, juvenile fish were reared in a recirculating aquaculture system and distributed into 18 tanks in total (n = 3 replicates per treatment, 40 fish per tank).

In Trial 1, fish (97.5 ± 17.7 g) received a 1-h bath treatment with one of two biocontrol products (10 g/m³) or remained untreated (control). After the bath treatment, fish were challenged by immersion with *A. veronii* (7 × 10⁵ CFU/mL) for 2.5 h and monitored for 2 weeks.

In Trial 2, fish (68.4 ± 12.3 g) were fed either a control diet or diets supplemented with one of two probiotics (1 g/kg of feed) during a 5-week nutritional preparation period. Fish were subsequently challenged by immersion with *A. veronii* (4 × 10⁵ CFU/mL) for 2.5 h and monitored for 2 weeks.

Results

Following *A. veronii* immersion challenge, both biocontrol treatments numerically increased survival relative to the control (1.9%), reaching 6.5% and 8.3% in the Biocontrol A and Biocontrol B groups, respectively. Although overall survival differences were not significant (Log-rank, p = 0.061), an ordered trend test (p = 0.023) indicated a graded treatment response (Control < Biocontrol A < Biocontrol B). These results suggest that bath-applied biocontrol treatments, especially Biocontrol B, may enhance seabass resilience to *A. veronii* infection.

In Trial 2, survival remained very low across all groups (0.9–2.9%), indicating a highly severe challenge. No significant differences in overall survival were detected among dietary treatments (Log-rank, p = 0.057), although early mortality dynamics differed significantly (Gehan–Breslow–Wilcoxon, p = 0.009). These results suggest limited effects of dietary supplementation on final survival under the challenge conditions tested.

Conclusion

Overall, the two challenge trials produced different protective outcomes against *A. veronii* infection in European seabass. In Trial 1, bath application of microbial biocontrol products numerically increased final survival and produced a significant positive treatment-related trend despite the severity of the challenge. In Trial 2, dietary probiotic supplementation did not significantly improve final survival, although it affected early mortality dynamics. These findings suggest that bath-applied

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microbial strategies may provide a rapid protective effect under acute challenge conditions. However, because the two trials differed in fish size, challenge dose, timing, and administration protocol, direct comparison between immersion and dietary administration should be interpreted cautiously. Future studies should evaluate combined immersion- and feed-based microbial strategies in a matched experimental design to determine whether complementary modes of action can improve resistance and survival following *A. veronii* infection. In addition, these microbial interventions could be explored as complementary tools alongside vaccination programs, including autogenous vaccines, particularly during periods of elevated disease risk or anticipated outbreaks. Such integrated prevention strategies may help reinforce host resilience and reduce disease impact under commercial farming conditions.

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EU AQUACULTURE: GREENHOUSE GAS REDUCTION COSTS AND PATHWAYS TO NET ZERO BY 2050

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The transition of European Union (EU) aquaculture toward carbon neutrality is critical for meeting climate targets while ensuring food security and sectoral sustainability. This study presents a comprehensive analysis of historic and predicted future greenhouse gas (GHG) emissions across five aquaculture production systems (cages, ponds, raceways, recirculating aquaculture systems, and non-fed systems). Emissions were analysed across the full value chain, including feed production and transport, energy use, logistics, and farm operations.

Eight future scenarios were developed to explore emission reduction pathways from 2025 to 2050, ranging from business-as-usual to maximum mitigation. These scenarios incorporate seven key mitigation tools, including renewable energy adoption, emerging low-carbon feed materials, artificial intelligence (AI)-based optimisation, integrated multi-trophic aquaculture (IMTA), energy efficiency improvements, system diversification, and expansion of non-fed aquaculture, alongside offsetting mechanisms such as carbon credits and ecosystem restoration.

Results indicate that feed production is the dominant emission source, accounting for approximately more than half of total GHG emissions, followed by energy-intensive grow-out systems, particularly in RAS. While technological and operational innovations can deliver substantial reductions, mitigation measures are insufficient to achieve net-zero emissions due to residual emissions and projected sector growth.

Marginal abatement costs (MACs) vary significantly across systems and time horizons, ranging from €46 to €243 per tCO₂eq (2026–2030) and stabilising at €69–€228 per tCO₂eq by 2050. Non-fed systems exhibit higher MACs due to initial lower emissions per tonne and the limited applicability of certain technologies, while fed systems rely heavily on upstream decarbonisation of feed and energy supply chains. Multi-criteria analysis (MCA), incorporating economic, environmental, and social dimensions, reveals that scenarios aligned with EU climate targets and those deploying a wide range of mitigation tools perform best overall, although economic constraints and regulatory barriers limit feasibility.

The findings highlight that achieving carbon neutrality in EU aquaculture requires a systemic approach combining full deployment of mitigation technologies, credible carbon offsetting, and coordinated decarbonisation of upstream sectors. Key policy priorities include accelerating renewable energy adoption, fostering innovation in feed and breeding, supporting digitalisation, reforming regulatory frameworks, and developing robust carbon accounting and crediting systems. Without such coordinated action, the sector is unlikely to meet its 2030–2050 climate targets.

Acknowledgment

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The information and views set out in this study are those of the author(s) and do not necessarily reflect the official opinion of CINEA or of the Commission. Neither CINEA nor the Commission can guarantee the accuracy of the data included in this study. Neither CINEA, the Commission or any person acting on their behalf may be held responsible for the use that may be made of the information contained therein.

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TRACKING BELOW THE SURFACE: UNCOVERING EUROPEAN SEABASS BEHAVIOUR WITH ACOUSTIC TELEMETRY

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Introduction

Ensuring fish welfare is a key aspect of modern aquaculture, as it impacts both ethical considerations and production performance. Environmental factors and routine operations on aquaculture farms can influence welfare status and lead to physiological and behavioural changes in fish, highlighting the need to assess both these responses. In this context, the development of welfare monitoring tools has become essential within the framework of precision aquaculture, facilitating decision-making on farms (Føre et al., 2017). Among the available technologies, acoustic telemetry allows the recording of fish behaviour under real farming conditions, such as in floating sea cages (Muñoz et al., 2020). However, most studies have focused on salmonids, and knowledge remains limited for Mediterranean species such as European seabass (*Dicentrarchus labrax*), particularly in open-sea net pens. Furthermore, hidden Markov models (HMMs) offer a useful tool for inferring behavioural states from telemetry data. These models have been widely used in wild fish populations, but their application in aquaculture studies remains limited. Consequently, the aim of this study is to analyse the behavioural patterns of seabass in open-sea net pens across different timescales, as well as to characterize their behavioural states in relation to environmental conditions using acoustic telemetry and HMMs.

Materials and Methods

The activity and vertical distribution of seabass were monitored in four experimental net-pens belonging to IRFAP-LIMIA (Spain). A total of 2,200 adult seabass (weight $\approx$ 1 kg) were distributed across the four cages, which measured 5.5 m in diameter and 6–8 m in depth ($N = 550$ individuals per cage). Five fish were randomly selected and implanted with acoustic transmitters (ADP-LP7; Thelma Biotel Ltd.). After 7 days of recovery in monitored indoor tanks, fish were returned to the cages, where seabass acceleration and depth were recorded for 5 months between July and November 2023. The data were recorded by 3 acoustic receivers (TBR 800; Thelma Biotel Ltd.) positioned between the cages. A total of ~ 1.2 million detections were processed. To account for structural differences between cages, depth was calculated relative to the centre of each cage. Linear mixed models (LMMs) and generalized additive models (GAMs) were applied to assess behavioural patterns and to explore the influence of environmental variables on the behaviour of seabass. To characterize behavioural states, a HMM with four states was developed: resting, deep swimming, surface swimming and high activity, estimating the transition probabilities between states whilst taking environmental covariates into account. All procedures were approved by the Animal Experimentation Ethics Committee (CEE-UIB, Spain; Ref. 206/12/22) and were carried out in accordance with European Directive 2010/63/EU and Royal Decree 53/2013.

Results and Discussion

Acoustic transmitters enabled a successful detection of swimming patterns and vertical distribution of seabass reared in open-sea net pens, which showed changes throughout the five-month experimental period. On one hand, swimming activity decreased slightly over the study period, whilst the fluctuations observed in vertical distribution proved to be sporadic, with the fish remaining stable most of the time in the middle zone of the water column. Clear circadian patterns were identified: swimming activity increased significantly during the day, peaking around midday, particularly after feeding. Vertical distribution also varied throughout the day, with greater use of deeper zones during the daylight hours and a greater presence at the surface at night. Furthermore, during the summer months, an increase in activity and greater use of depth were observed. GAM models revealed a significant effect of environmental variables on the behaviour of seabass. Solar radiation showed a strong positive correlation with both parameters, and wave height and wind also affected vertical distribution. The HMM allowed the identification of the four behavioural states and the characterization of their dynamics throughout the day. A clear daily pattern was observed: at night, resting and surface swimming predominated, whereas deep swimming increased after dawn. Subsequently, following feeding, deep swimming behaviour decreased, giving way

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to an increase in high-activity behaviour, which peaked 3–4 hours after feeding. At dusk, a transition towards lower-activity states was observed. Transitions between states were strongly modulated by solar radiation and the daily cycle, which favoured the shift from low- to high-activity states and promoted the occurrence of active states during daylight hours. Taken together, these results are consistent with previous studies indicating that seabass behaviour in open-sea net pens is strongly regulated by environmental factors and daylight cycle (Toomey et al., 2025). The integration of acoustic telemetry and machine learning models allows for the characterization of behavioural states and their dynamics under farming conditions. This approach offers a robust framework for improving welfare monitoring and promoting welfare-oriented management of open-sea aquaculture facilities.

Acknowledgment

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PRELIMINARY EVALUATION OF STRUCTURAL AND OCCUPATIONAL ENVIRONMENTAL ENRICHMENT IN FARMED RAINBOW TROUT

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Introduction

Aquaculture is a rapidly growing animal production sector, but intensive farming conditions may challenge fish welfare by limiting environmental complexity and natural behaviours. Environmental enrichment can increase habitat complexity and promote natural activity, although its application in commercial aquaculture remains limited and its effects depend on the species and enrichment strategy (Arechavala-Lopez, et al., 2022).

This study evaluated the effects of two environmental enrichment strategies on rainbow trout (*Oncorhynchus mykiss*): structural enrichment using vertically suspended PVC tubes and occupational enrichment using additional water currents. Their effects were assessed through animal-based welfare and physiological indicators, including external lesions, body condition, hepatosomatic index, muscle pH, skin and muscle colour, and muscle glycogen.

Materials and Methods

A total of 800 triploid rainbow trout (*Oncorhynchus mykiss*; 15–20 g) were randomly allocated to three treatments (4 tanks/group; 50 fish/tank): Control, without enrichment; Structural enrichment, with vertically suspended PVC tubes; and Occupational enrichment, with additional water currents generated by submersible pumps for 12 h/day. Fish were fed commercial feed (1% body mass/day) and maintained under experimental conditions for 3 months.

At the end of the trial, welfare and post-mortem indicators were evaluated, including body condition, external lesions, hepatosomatic index (HSI), muscle pH (0 and 24 h), skin and muscle colour (L*, a*, b*), and muscle glycogen. Data were analysed using ANOVA, repeated-measures analysis for muscle pH, and Chi-square tests for lesions. Bonferroni tests were used for post-hoc comparisons, with significance set at $p < 0.05$.

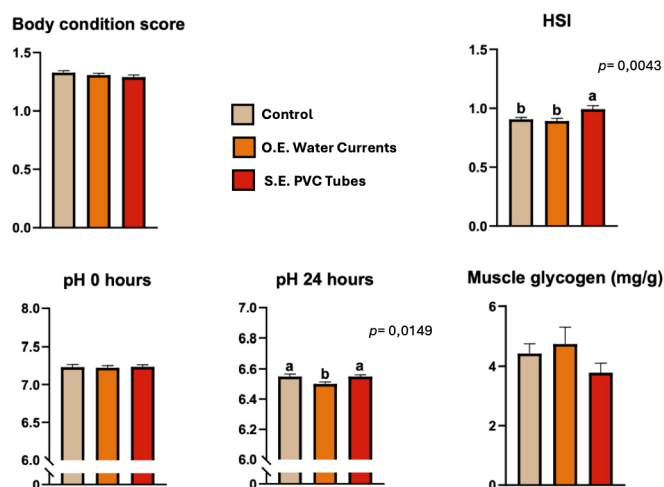


Figure 1 Mean $\pm$ SEM (Standard error of means) of body condition score, hepatosomatic index (HSI), muscle pH at 0 and 24 h *post-mortem*, and muscle glycogen content in rainbow trout from the control group and the two environmental enrichment treatments: occupational enrichment (O.E. water currents) and structural enrichment (S.E. PVC tubes). Different letters indicate significant differences ($p < 0.05$).

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Results

External lesions were generally low across all experimental groups, with no significant differences among treatments. Similarly, body condition, skin and muscle colour (L^* , a^* , b^*), and muscle glycogen content at slaughter showed no significant differences between the control and enriched groups.

Significant differences were observed in the hepatosomatic index (HSI), with fish exposed to structural enrichment (PVC tubes) showing higher HSI values than both the control and occupational enrichment (water currents) groups ($p = 0,0043$). Muscle pH was similar among treatments immediately after slaughter (0 h; approximately 7,2), but significant differences were detected after 24 h ($p = 0,0149$). Fish exposed to occupational enrichment showed lower muscle pH values than both the control and structural enrichment groups, while no differences were observed between the latter two groups.

Discussion

Environmental enrichment with suspended PVC tubes or additional water currents did not negatively affect the external appearance, body condition, skin or muscle colour, or muscle glycogen content of rainbow trout, suggesting that both strategies were compatible with fish welfare under the experimental conditions (Crank et al., 2019). The absence of differences in external lesions indicates that the suspended tubes did not represent an additional source of physical damage, while the similar body condition among groups suggests no major effect on overall growth or physical status. However, structural enrichment resulted in a higher hepatosomatic index, which may reflect differences in energy storage or metabolic status (De la Llave-Propín et al., 2024). Occupational enrichment with water currents also affected muscle pH 24 h post-mortem, with lower values than those observed in the control and structural enrichment groups, potentially related to increased swimming activity and pre-slaughter muscle metabolism. Nevertheless, these differences were moderate and were not accompanied by changes in muscle glycogen. Overall, the results suggest that environmental enrichment may influence specific physiological indicators without negatively affecting general fish condition, although further studies integrating behavioural, productive, and metabolic indicators are needed to better assess its potential benefits for rainbow trout welfare.

Acknowledgements

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***Vibrio harveyi* ISOLATES AND THEIR EXTRACELLULAR PRODUCTS DIFFERENTIALLY MODULATE THE RESPONSES OF EUROPEAN SEA BASS PERIPHERAL BLOOD LEUKOCYTES**

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Background

Aquaculture is the fastest growing food-production sector and plays a major role in providing high-quality animal proteins for the growing global population. Nevertheless, aquaculture production faces numerous challenges, particularly infectious diseases, whose emergence is becoming more prevalent with climate change. One emerging pathogen in the Mediterranean aquaculture is *Vibrio harveyi*, a free-living Gram-negative bacterium, that can cause disease outbreaks in a range of organisms, including fish, crustaceans, molluscs, corals, sponges and algae. In addition, *V. harveyi* has been associated with human infections, showing zoonotic potential. The pathogenicity of *V. harveyi* varies among strains, with some strains causing high mortality rates, while others appear non-virulent and may occur as part of the intestinal microbiota of healthy fish. The aim of this work was to assess the immune responses of European sea bass peripheral blood leukocytes (PBLs) following stimulation with two strains of *V. harveyi*, one isolated from microbiota of healthy fish and the other recovered from a clinical case of disease, as well as their extracellular products (ECPs).

Methodology

Bacterial strains were grown overnight in Marine Broth, pelleted by centrifugation, and resuspended in phosphate-buffered saline (PBS). The corresponding culture supernatants were retained for recovery of extracellular products (ECPs) using ultracentrifugation columns. European sea bass blood was collected in heparinised tubes, diluted with incomplete Leibovitz L15 medium and layered onto 51% iso-osmotic Percoll to isolate PBLs. Isolated PBLs were seeded in 6-well plates in complete L15 medium and stimulated for 4, 8 and 24h with two strains of *V. harveyi* and their respective ECPs. Unstimulated cells were included as negative controls, whereas cells stimulated with lipopolysaccharide (LPS) served as positive controls. Stimulation was terminated by resuspending the cells in TRIReagent. Total RNA was extracted, reverse-transcribed and used to quantify the expression of a panel of immune-related genes, including pro-inflammatory cytokines (*Il1b*, *Il6*, *Il8*, *Tnfa*), anti-inflammatory cytokine *Il10*, acute-phase proteins and antimicrobial peptides (*Cox2*, *Leap/Hepcidin*, *Lyz*), adaptive immunity markers (*Ifng*, *Il4/Il13*, *Il17*), apoptosis marker *Casp3*, with *Efla*, *rna18s* and *actb* as housekeeping genes.

Results

Preliminary analyses are ongoing. We expect that *V. harveyi* isolates and their extracellular ECPs will differentially modulate immune-gene expression in European sea bass PBLs in a time-dependent manner. The isolate recovered from a clinical disease case and its ECPs may induce a stronger pro-inflammatory response than the isolate originating from the microbiota of healthy fish. These results will contribute to understanding strain-specific immunomodulatory effects of *V. harveyi* and its ECPs in European sea bass.

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IMPACT OF ENVIRONMENTAL STRESSORS ON PHYSIOLOGICAL STATUS AND IMMUNE RESPONSES OF WHITELEG SHRIMP *Litopenaeus vannamei*

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Introduction

Whiteleg shrimp *Litopenaeus vannamei* is one of the most economically significant species in global aquaculture. It is primarily farmed in ponds which are increasingly threatened by extreme weather induced by climate change (Galappaththi *et al.*, 2020). Whilst the effects of individual stressors have been documented (Zheng *et al.*, 2017; Wang *et al.*, 2020), the physiological impact of combined environmental shifts, such as rapid changes in temperature and salinity caused by heatwaves and heavy rainfall, remains poorly understood. These stressors may induce physiological stress, leading to disrupted immune regulation and microbiome dysbiosis. As a result, this study hypothesises that the synergistic effect of these stressors would induce acute oxidative stress and compromise mucosal barrier integrity, leading to systemic physiological instability and extensive lipid peroxidation within haemocytes and immune-related tissues. Therefore, this study aimed to investigate the effects of extreme environmental shifts on the mucosal immunity of *L. vannamei*.

Materials and Methods

Early juvenile *Litopenaeus vannamei* (mean weight: 0.20 ± 0.10 g) were acclimatised in a recirculating aquaculture system (27°C, 25 ppt) for two weeks before being randomly allocated into three groups in quadruplicate: 1] high temperature and high salinity group (HTHS: 33°C, 40 ppt), 2] low temperature and low salinity group (LTLS: 20°C, 10 ppt), and 3] a control group (27°C, 25 ppt). Environmental parameters were adjusted during a three day ramping phase at a rate of change of 3°C and 5 ppt per day. The sampling regime comprised nine shrimp per group, sampled across the four replicate tanks with 2-3 shrimp per tank at each sampling point. Sampling points were on the day before the ramping phase (T0), and at 24 hours (T1) and 5 days (T2) after achieving the target temperature and salinity. Haemolymph was extracted using a syringe pre-loaded with anticoagulant, and haemocytes were subsequently collected via centrifugation at $800 \times g$ for 30 minutes at 4°C. The cell pellets were then lysed in 1/3 PBS to quantify the activities of lysozyme (LYZ) and superoxide dismutase (SOD), and the levels of malondialdehyde (MDA), using commercial assay kits (Abcam, Cambridge, UK) according to the manufacturer's instructions. Statistical significance was determined using the Kruskal-Wallis test followed by Dunn's *post-hoc* test with Bonferroni correction for multiple comparisons ($P < 0.05$), analysed using the R software environment. Furthermore, gills and intestines were preserved in ethanol for microbiome analysis while gills, intestines, and hepatopancreas were excised for immune gene expression analysis via RT-qPCR.

Results

At the initial sampling points, no significant differences in the biochemical markers were observed between the control and the stress treatment groups ($P > 0.05$). In contrast, at T2, the lysozyme activity (Figure 1A) in the HTHS group (0.87 ± 0.19 mU mg⁻¹) was significantly higher than that of the control group (0.35 ± 0.06 mU mg⁻¹) ($P = 0.03$). Similarly, SOD activity (Figure 1B) was significantly higher in the LTLS group (566.70 ± 133.03 U mg⁻¹) compared to the control group (282.08 ± 35.24 U mg⁻¹) ($P = 0.01$). Most notably, MDA levels (Figure 1C) in both the HTHS (2.40 ± 0.70 nmol mg⁻¹) and LTLS (3.45 ± 1.31 nmol mg⁻¹) groups were significantly higher than the control group (0.38 ± 0.04 nmol mg⁻¹) ($P = 0.0018$ & 0.0001 , respectively).

Discussion

These findings indicate that combined temperature and salinity shifts pose a severe physiological burden on early juvenile *L. vannamei*. Particularly, the significant elevation in MDA levels within the LTLS group indicates lipid peroxidation, suggesting that oxidative stress has exceeded the host's antioxidant capacity (Li *et al.*, 2017). Whilst significant increases in SOD and lysozyme activities reflect an active mobilisation of the antioxidant defence and innate immunity, such a prolonged response may impose a substantial metabolic cost. This may confer a metabolic trade-off between immune

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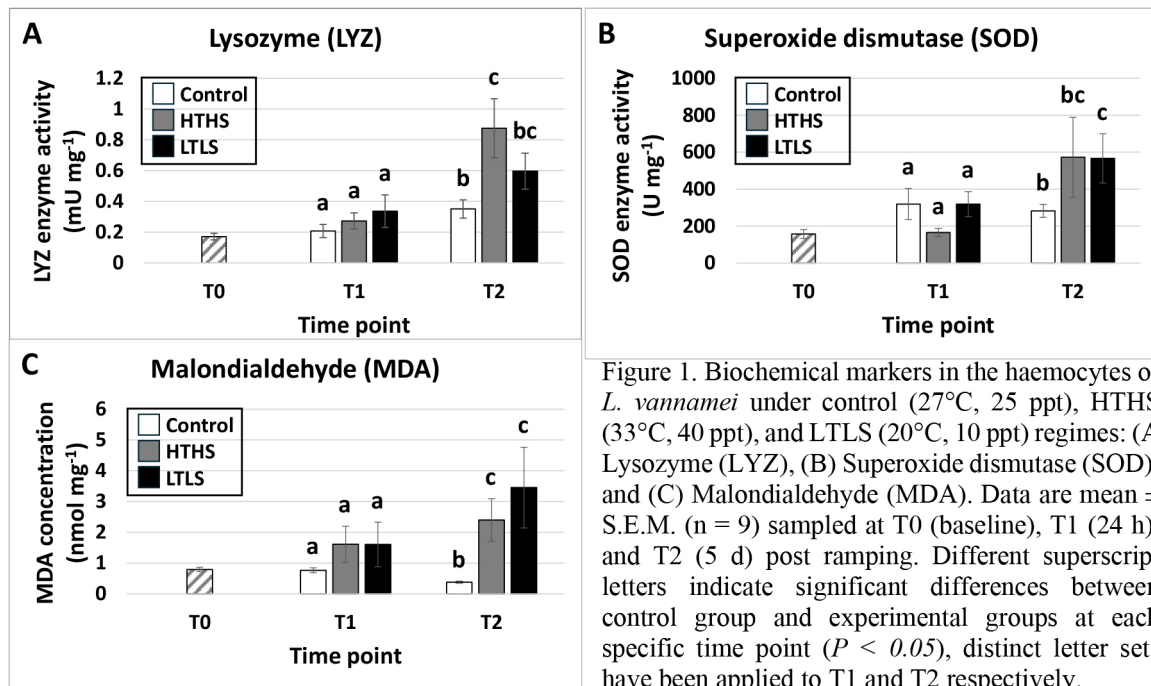


Figure 1. Biochemical markers in the haemocytes of *L. vannamei* under control (27°C, 25 ppt), HTHS (33°C, 40 ppt), and LTLS (20°C, 10 ppt) regimes: (A) Lysozyme (LYZ), (B) Superoxide dismutase (SOD), and (C) Malondialdehyde (MDA). Data are mean $\pm$ S.E.M. (n = 9) sampled at T0 (baseline), T1 (24 h), and T2 (5 d) post ramping. Different superscript letters indicate significant differences between control group and experimental groups at each specific time point ($P < 0.05$), distinct letter sets have been applied to T1 and T2 respectively.

tolerance and physiological state. These results show the vulnerability of early-stage shrimp to simulated extreme weather events. Ongoing microbiome profiling, integrated with host immune gene expression analyses, will clarify whether these biochemical alterations are associated with intestinal dysbiosis and concomitant modulation of specific immunometabolic pathways. This integrative approach will provide mechanistic insight into host-microbe-immune interactions, thereby informing the development of targeted intervention strategies to enhance immune competence and resilience in commercial aquaculture systems.

Figures

Acknowledgment

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STRESS RESPONSE OF THREE *Mx* GENES IN GIANT GROUPER (*epinephelus lanceolatus*)

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Introduction

The giant grouper (*Epinephelus lanceolatus*) is a cornerstone of Taiwan's aquaculture industry; however, infection by nervous necrosis virus (NNV) during the larval and juvenile stages results in mortality rates as high as 90%. Consequently, elucidating the host immune mechanisms following viral infection is critical for developing effective preventive strategies. Myxovirus resistance (Mx) proteins are key downstream effectors of the type I interferon (IFN) system. Although our previous research demonstrated that *ggMx1,2, 3* mRNA levels are regulated by NNV, it remains unclear whether these three Mx isoforms possess distinct temporal roles in inhibiting NNV replication, and the precise regulatory mechanisms of their respective proteins have yet to be fully clarified. In this study, we characterized the distribution and dynamic shifts of the Mx proteins under viral stress.

Materials and Methods

GF-1 cells were utilized as the experimental model. The overexpression of Mx1, 2, 3 proteins were characterized under NNV challenge. Subcellular distribution and dynamic protein shifts were visualized using high-resolution confocal immunofluorescence imaging. To investigate physical interactions between the host immune factors and the pathogen, colocalization analysis was performed between Mx proteins and the NNV capsid protein.

Results

Confocal immunofluorescence imaging revealed the distribution and dynamic shifts of Mx proteins, demonstrating that following the overexpression of Mx1, 2, 3 the peak suppression of NNV occurred sequentially, starting with Mx1 protein, followed by Mx2 protein, and finally Mx3 protein. Colocalization analysis confirmed physical interactions between Mx proteins and the NNV capsid protein, with peak colocalization observed at 30 hours for Mx1, 2, and 36 hours for Mx3.

Discussion

This study provides novel insights into the temporal and spatial regulation of the innate immune response in giant grouper. The sequential peak suppression of NNV by different Mx homologs suggests a coordinated, stage-specific antiviral defense strategy. The physical interaction and peak colocalization times indicate that Mx1 and Mx2 act earlier in the infection cycle compared to Mx3. Collectively, these findings elucidate the dual response mechanisms of *ggMx* to biological and viral stress, providing profound insights into the interaction of NNV and Mx and supporting the development in aquaculture.

Acknowledgment

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USING ALTERNATIVE FEED SOURCES FROM AGRICULTURAL BY-PRODUCTS TO REPLACE FISHMEAL: ADDRESSING CHALLENGES FOR AQUACULTURE SUSTAINABILITY

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Introduction

Aquaculture is the fastest-growing global food production sector and is vital for food and nutritional security. With output reaching approximately 87.5 million tonnes, over 70% of production relies on formulated feeds. Fish meal (FM) has traditionally been the primary protein source due to its amino acid profile, digestibility, and palatability (Luthada-Raswiswi et al., 2021). However, its limited supply from capture fisheries and rising demand have caused price volatility and supply concerns, making sustainable FM alternatives an urgent priority. Plant-based proteins are promising substitutes due to their abundance, lower cost, renewability, and reduced environmental impact. Ingredients such as soybean meal, *Moringa oleifera* leaf and seed meal, canola meal, sunflower meal, and cottonseed meal are already used in commercial aquafeeds. Yet, antinutritional factors (ANFs) including phytate, tannins, saponins, protease inhibitors, and non-starch polysaccharides limit their inclusion by reducing nutrient bioavailability and impairing fish growth and health. Feed additives such as exogenous enzymes, probiotics, prebiotics, antioxidants, organic acids, and functional nanoparticles can mitigate ANF effects, improve digestibility, and enhance fish health, allowing higher plant protein incorporation. Nevertheless, further research is needed on the long-term physiological, economic, and environmental impacts of large-scale FM substitution. Species-specific formulations, life-cycle assessments, and cost-benefit analyses remain critical. Developing eco-efficient, nutritionally complete, and cost-effective aquafeeds is essential for sustainable aquaculture growth and global food security (Hussain et al., 2024).

Acknowledgment

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ESTABLISHMENT OF REPRODUCIBLE MULTIFACTORIAL CHALLENGE MODELS FOR WHITE FAECES DISEASE IN SHRIMP

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Introduction

Global shrimp aquaculture has expanded rapidly over the past decades, driven by increasing demand and the widespread adoption of intensive and super-intensive farming systems. Production of *Penaeus vannamei* now dominates global shrimp output, particularly in Asia. However, high stocking densities and suboptimal environmental control have increased disease outbreaks, significantly affecting productivity.

White Faeces Disease (WFD) has emerged as a major challenge, characterized by floating white fecal strings and reduced growth performance. It is widely considered a multifactorial disease, with the microsporidian *Ecytonucleospora hepatopenaei* (EHP) identified as a primary pathogen causing hepatopancreatic dysfunction. Opportunistic *Vibrio* spp., including *V. parahaemolyticus* and *V. alginolyticus*, are thought to act as secondary agents that exacerbate disease severity. Despite its impact, the lack of a reproducible challenge models limit understanding of WFD pathogenesis and the development of effective treatments. This study aims to develop a standardised co-infection model to better understand WFD and support disease management strategies. This approach provides, for the first time, a controlled framework to reproduce WFD under experimentally defined multifactorial conditions.

Approach

To ensure the acquisition of high-quality biological materials, a collaborative effort was established between IMAQUA B.V. (Belgium), Ghent University (Belgium), and Can Tho University (Vietnam). Field surveys and targeted sampling campaigns were conducted in shrimp farming regions affected by WFD and confirmed EHP presence. Diseased shrimp specimens were collected for pathogen isolation and characterization.

Bacterial isolates were obtained from hepatopancreatic and intestinal tissues using selective culture techniques. Approximately 20 *Vibrio* strains representing multiple species, including *V. parahaemolyticus*, *V. alginolyticus*, and *V. vulnificus* were successfully isolated. At the Laboratory of Aquaculture and Artemia Reference Center (ARC), these strains underwent comprehensive characterization, including molecular identification via 16S rRNA gene sequencing. Functional assays were conducted to assess virulence-related traits, such as quorum sensing activity and bacterial motility.

In parallel, EHP spores were isolated from infected shrimp at Can Tho University (Vietnam) and propagated under controlled laboratory conditions at IMAQUA. Successful experimental transmission of EHP was achieved, confirming the viability of the spores and enabling their use in subsequent co-infection trials.

This integrated pipeline from field sampling to laboratory validation ensures that both primary and secondary pathogens used in this study reflect real-world disease dynamics while maintaining experimental reproducibility.

Discussion

This initial phase successfully established a high-quality pathogen collection representing both primary (EHP) and secondary (*Vibrio* spp.) agents of WFD. These results provide a critical foundation for developing a controlled co-infection model.

Future work will focus on optimizing challenge protocols by varying pathogen concentrations and exposure timing to reliably reproduce WFD symptoms. Such a model will enable deeper investigation of pathogen interactions and provide a robust platform for evaluating therapeutics and management strategies.

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Developing a reproducible WFD model represents an essential step toward transforming a complex field syndrome into a predictable and manageable disease, ultimately supporting the sustainability of shrimp aquaculture.

Overall, this study establishes the foundation for a co-infection model of White Faeces Disease by integrating field-derived EHP and *Vibrio* isolates with laboratory infection systems. The upcoming optimization of this model is expected to enhance understanding of disease mechanisms and facilitate the development of effective control strategies in shrimp farming.

Acknowledgment

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EFFECT OF DILUTION RATIOS ON THE POST-THAW MOTILITY AND FERTILIZING CAPACITY OF CRYOPRESERVED *Heterobranchus longifilis* SPERM

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Introduction

Cryopreservation is an important reproductive biotechnology used for the long-term preservation of fish sperm at ultra-low temperatures without significant loss of fertilizing ability (Tiersch et al., 1994). In aquaculture, sperm cryopreservation supports selective breeding, synchronization of gamete availability, conservation of valuable genetic resources, and year-round seed production. *Heterobranchus longifilis* is an economically important African catfish species widely used in hybridization programmes because of its fast growth and aquaculture potential. Successful cryopreservation of fish sperm is influenced by several factors, including extender composition, cryoprotectant type, freezing conditions, and sperm dilution ratio (Horváth et al., 2001). Appropriate dilution ratios are essential to maintain sperm quality during freezing and thawing by reducing osmotic and cryoinjury effects. Therefore, this study evaluated the effect of different dilution ratios on the post-thaw motility and fertilizing capacity of cryopreserved *Heterobranchus longifilis* sperm.

Materials and Methods

Heterobranchus longifilis broodstock was maintained at the Research Center for Fisheries and Aquaculture in Szarvas, Hungary. Testes of sexually mature males were excised following hormonal stimulation of spermatogenesis and overanesthesia. Testes were collected at two different dates. Sperm was extracted from the testicular tissue and diluted in either carp extender (200 mM glucose, 40 mM KCl, 30 mM Tris, pH 8.0) or catfish extender (6% fructose, 20 mM NaHCO₃) supplemented with 10% methanol as a cryoprotectant. Sperm was diluted to 0.5, 1 or 2 × 10⁹ cells per ml or alternatively to a dilution ratio of 1:1 and cryopreserved in the vapor of liquid nitrogen. Post-thaw motility was determined by computer-assisted sperm analysis (CASA). Fertilizing capacity of frozen-thawed sperm was assessed using the eggs of *Clarias gariepinus* 24 h after fertilization.

Results

Progressive motility of cryopreserved *H. longifilis* sperm was low (below 12% during the first collection date and below 2% in the second). At the first collection date both the extender ($P = 0.009$) and the dilution ratio ($P = 0.001$) had a significant effect on the post-thaw motility. The highest motility was observed using carp extender with sperm diluted to 1 × 10⁹ cells per ml (12 ± 8%). Generally, dilution of sperm to 0.5 × 10⁹ cells per ml resulted in significantly lower post-thaw motility (Figure 1A). At the second collection date, dilution of sperm to a dilution ratio of 1:1 resulted in the highest post-thaw motility (2 ± 1%). Dilution of sperm also had a significant effect on the fertilizing capacity of *H. longifilis* sperm ($P < 0.001$) with dilution ratios of 1 and 2 × 10⁹ cells per ml as well as 1:1 resulting in higher fertilization than dilution to 0.5 × 10⁹ cells per ml (Figure 1B).

Acknowledgment

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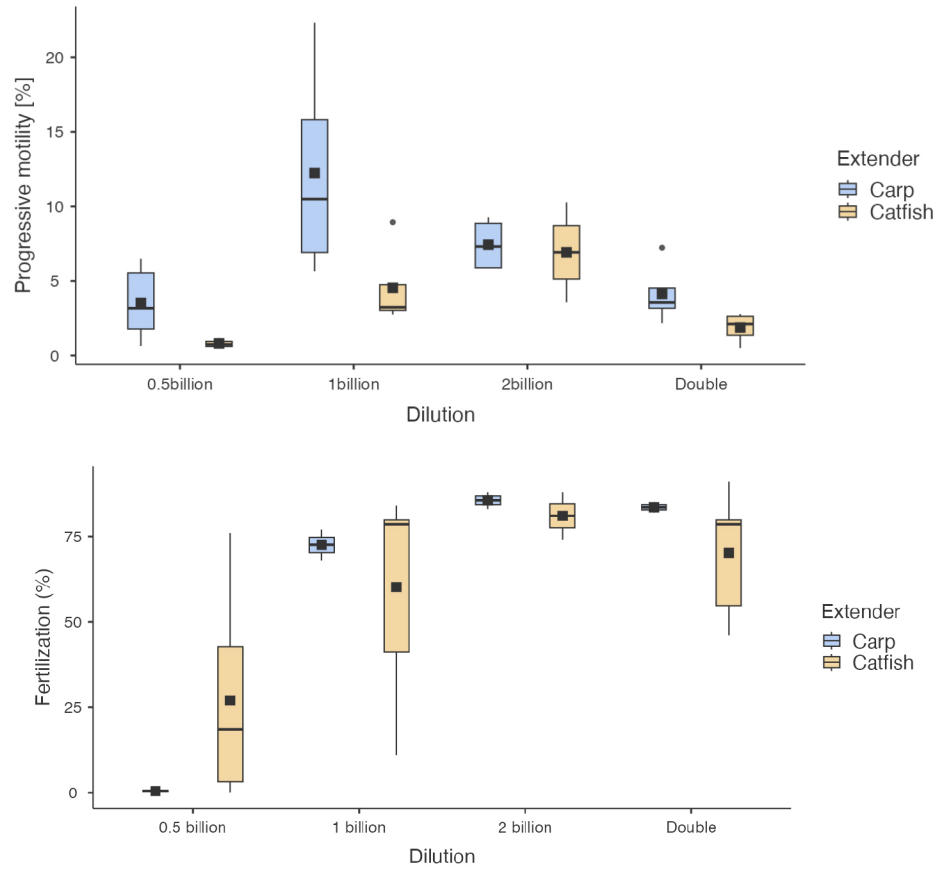


Figure 1: Post-thaw motility (top) and fertilizing capacity (bottom) of cryopreserved *Heterobranchus longifilis* sperm. Sperm was cryopreserved in using either carp or catfish extenders and diluted to to 0.5, 1 or 2×10^9 cells per ml or alternatively to a dilution ratio of 1:1 (labeled as Double)

FULL-COVERAGE MONITORING OF FISH DISTRIBUTION IN AQUACULTURE SEA CAGES USING A DUAL TWO-DIMENSIONAL SONAR COMBINATION SYSTEM

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Introduction

Monitoring fish behaviour, such as swimming speed and spatial distribution, in aquaculture sea cages is essential for optimizing feeding management and improving fish welfare. Stereo cameras have been widely used to measure swimming speed; however, they rely on machine-learning-based image processing, which demands substantial computational resources and power, and their performance is degraded by low illumination and biofouling of the camera housing (Ikegami et al., 2026). In addition, stereo cameras provide only a local field of view and cannot capture the spatial distribution of fish across the entire cage. Multibeam sonars offer a light-independent alternative, but their limited field of view makes it difficult to cover an entire commercial sea cage with a single unit. Placing the sonar near the cage wall extends the observable area but introduces detection-range limitations (Banno et al., 2025). A mechanically rotating single-beam sonar overcomes both range and field-of-view limitations by scanning 360° from the cage centre (Sauphar et al., 2026); however, the rotation mechanism limits temporal resolution, and swimming speed cannot be derived from this approach.

In this study, we present a dual two-dimensional (2D) sonar combination system that addresses both the field-of-view and the real-time monitoring challenges simultaneously. Each sonar unit has a fan-shaped beam with an opening angle of 120°. By orienting two units perpendicular to each other, the system achieves near-complete coverage of the cage cross-section without any mechanical rotation, enabling continuous monitoring of swimming speed and depth distribution. Furthermore, all the approaches mentioned above provide only two-dimensional observations, whereas Atlantic salmon (*Salmo salar*) are known to exhibit three-dimensional spatial heterogeneity within cages. To address this, we additionally conducted a preliminary verification of three-dimensional (3D) monitoring using a multibeam sonar equipped with a rotational scanning mechanism.

Materials and Methods

Two field experiments were conducted. The dual 2D sonar experiment was carried out in September 2025 at the NTNU salmon aquaculture research site Gudmundset, on the west coast of Norway, in a commercial sea cage containing Atlantic salmon post-smolts. Two multibeam sonars (FCS-500, FURUNO ELECTRIC, Japan; 120° beam opening angle per unit) were deployed as a twin unit at the centre of the cage just below the water surface, with their fan beams oriented perpendicular to each other; this configuration constitutes the dual 2D sonar combination evaluated here. Recordings were obtained continuously over extended observation periods covering different times of day. From the calibrated volume backscattering strength (Sv), fish density was mapped on the depth–time plane and individual fish were tracked frame-to-frame to estimate the swimming speed. The 3D verification experiment was conducted in December 2025 in a commercial sea cage at Tokushima, Japan, containing yellowtail (*Seriola quinqueradiata*); a multibeam sonar equipped with a rotational scanning mechanism was installed at the cage centre, and the transducer was automatically rotated to produce three-dimensional snapshots of the spatial distribution of fish within the cage volume.

Results and Discussion

The dual 2D sonar system successfully captured spatial and temporal variations in fish distribution across the cage. Both swimming speed and depth distribution showed clear changes depending on the time of day, demonstrating the system's capability to quantitatively track diurnal behavioural patterns in real time under practical farming conditions. The estimated mean swimming speeds were approximately 0.6 m/s during daytime and 0.3 m/s during night-time. The rotational multibeam sonar confirmed the feasibility of capturing the volumetric distribution of fish within the cage.

The proposed dual 2D sonar system provides a practical, cost-effective, and real-time solution for wide-area behavioural monitoring in commercial sea cages, overcoming the key limitations of existing methods. Further integration of the dual 2D system with the 3D rotational scanning approach is expected to enable comprehensive whole-cage monitoring that accounts for the three-dimensional heterogeneity of fish distribution. Future work will include verification of the 3D approach with Atlantic salmon.

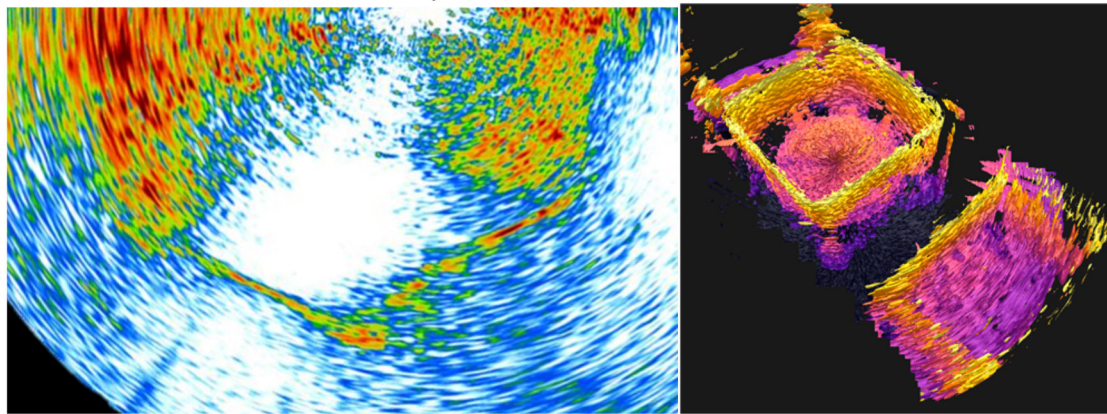
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Figure

The echo of the dual 2D sonar and 3D system.



AQUACULTURE VALUE CHAIN AND THE RESILIENCE LEVEL OF AQUA FARMERS TO CLIMATE CHANGE IN DELTA STATE, NIGERIA

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Introduction

Aquaculture contributes significantly to food security, employment, and economic development in Nigeria. However, climate change has increasingly threatened the sustainability of aquaculture production through flooding, salinity intrusion, disease outbreaks, and declining water quality. These challenges affect the entire aquaculture value chain and the resilience of aqua farmers. Despite the importance of aquaculture in Delta State, there is limited empirical evidence regarding the resilience of aquaculture value chain actors to climate change and the influence of gender on adaptive capacity. This study therefore assessed the aquaculture value chain and evaluated the resilience levels of male and female aqua farmers to climate change in Delta State, Nigeria.

Materials and Methods

The study was conducted in Delta State, Nigeria, using a purposive sampling technique. A total of 991 aqua farmers representing 30% of registered aquaculture farmers from identified clusters were selected. Primary data were collected through structured questionnaires and unstructured interviews. Data were analysed using descriptive statistics and t-test analysis with the aid of Statistical Package for Social Sciences (SPSS) version 24. Resilience was evaluated based on four dimensions: institutional, ecosystem, community, and economic resilience.

Results and Discussion

The socioeconomic characteristics showed that the majority of the respondents were male (57.1%), married (53.2%), and had attained formal education. The mean age of respondents was 42 years, while the average household size and aquaculture experience were six persons and sixteen years, respectively. The average monthly income from aquaculture was ₦137,492.93, indicating that aquaculture provides substantial economic benefits to practitioners.

The aquaculture value chain comprised input suppliers, breeders, table-size producers, marketers, processors, transporters, distributors, and consumers. Table-size producers constituted the largest proportion (40.5%) of value chain actors.

Assessment of resilience revealed gender differences in adaptive capacity to climate change. Male aqua farmers demonstrated community resilience, particularly through support from traditional leadership and local communities, but showed low resilience in institutional, ecosystem, and economic dimensions. Conversely, female aqua farmers exhibited greater economic resilience due to increased access to financial support programmes and empowerment initiatives but had lower resilience in institutional, ecosystem, and community dimensions.

The t-test analysis indicated a statistically significant difference ($p < 0.01$) between the resilience levels of male and female aqua farmers, leading to the rejection of the null hypothesis of no gender difference in resilience.

Conclusion

The study demonstrated that aquaculture in Delta State is supported by a diverse value chain involving multiple stakeholders. Significant gender disparities exist in resilience to climate change, with female aqua farmers exhibiting higher overall resilience than their male counterparts, largely due to greater economic empowerment opportunities. The findings highlight the need for gender-responsive policies and targeted interventions aimed at strengthening institutional, ecosystem, community, and economic resilience among aquaculture value chain actors. Such interventions are essential for promoting sustainable aquaculture development and enhancing the adaptive capacity of farmers to climate change in Delta State, Nigeria.

Table 1. Overall Resilience Level of Aqua Farmers by Gender

Resilience Level	Male (%)	Female (%)
Low Resilience (0–49%)	67.7	44.5
High Resilience (50–100%)	32.3	55.5

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PROSPECTS AND CHALLENGES OF CAPTURE-BASED AQUACULTURE FOR THE EUROPEAN EEL (*Anguilla anguilla*) IN TÜRKİYE: FROM LAGOON HARVESTING TO EXPORT

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The European eel (*Anguilla anguilla*) is one of the most economically valuable species in global aquaculture. Due to the biological constraints preventing closed-cycle artificial reproduction on a commercial scale, the industry relies entirely on rearing wild-caught individuals, a practice known as capture-based aquaculture. Türkiye, with its extensive coastal lagoon systems, holds significant potential for this sector. Currently, eel production and export in Türkiye primarily depend on harvesting from key lagoons such as Köyceğiz, Bafa, Paradeniz and Akyatan. The very few specialized facilities operate strictly on the principle of fattening individuals collected from these natural habitats.

However, the development and survival of the sector are directly dependent on strict international trade regulations and annually determined national export quotas. This study evaluates the primary bottlenecks facing eel aquaculture and lagoon management in Türkiye. These challenges include: (1) fluctuations in wild stock recruitment exacerbated by increasing water pollution, habitat destruction, the pressure of proliferating invasive species in lagoon lakes, and the potential occurrence of harmful algal blooms; and (2) the inherent technical difficulties of eel aquaculture operations coupled with a significant lack of qualified experts and specialized personnel in this specific field.

Furthermore, a critical vulnerability in the sector is the substantial lack of knowledge regarding fish health and diseases. While the presence of the invasive nematode *Anguillicoloides crassus* and certain common parasites is documented, epidemiological data on bacterial and viral diseases affecting Turkish eel populations remain highly insufficient. This lack of data poses severe risks, particularly during intensive fattening periods and export stages. Ultimately, ensuring the sustainability of the eel sector in Türkiye necessitates establishing ecologically based quotas, rigorous monitoring of water quality in lagoons, and the urgent development of aquaculture-focused health management strategies.

EFFECTS OF MONO- AND BINARY MICROALGAL ENRICHMENTS ON THE BIOCHEMICAL COMPOSITION OF THE CYCLOPOID COPEPOD *Apocyclops panamensis*

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Introduction

Copepods, which constitute a major component of the natural prey of marine fish larvae, represent a valuable live-feed option for ornamental fish larviculture. Live microalgae are essential for their culture and nutritional enrichment (Rasdi & Qin, 2016). Although most studies have focused on calanoid (Martino *et al.*, 2023, 2024) and harpacticoid copepods (Hansen *et al.*, 2018), the cyclopoid *Apocyclops panamensis* (Marsh, 1913) is receiving increasing attention as live prey for marine larviculture (Ballesteros-Redondo *et al.*, 2023). In this study, we evaluated the effects of three microalgal species, supplied individually or as binary combinations, on the proximate composition and fatty acid profile of *A. panamensis* after 24 h of enrichment.

Materials and Methods

The marine microalgae *Rhodomonas salina* (R), *Isochrysis galbana* (I) and *Chaetoceros calcitrans* (C) were used as enrichment diets. Copepods (*A. panamensis*) were randomly allocated to six experimental enrichment treatments: three monoalgal diets consisting of R, I or C, and three binary diets consisting of equal proportions (1:1) of RI, CI or RC. Copepods cultured on *Tetraselmis suecica* served as the control. Each treatment was performed in triplicate (n = 3), with each tank considered an independent experimental unit. After 24 h, the entire content of each tank was harvested, lyophilized, and subsequently analysed. Proximate composition and fatty acid profiles of copepods were analyzed. Moisture, protein and lipids were determined by thermal dehydration, Kjeldahl and Folch methods, respectively, while carbohydrates were calculated by difference. Fatty acid methyl esters (FAMES) were analyzed by gas-liquid chromatography following transmethylation. Treatment differences were assessed by ANOVA–Tukey or Welch’s ANOVA–Games–Howell according to variance homogeneity ($\alpha = 0.05$).

Results

After 24 h of enrichment lipid content differed significantly among treatments ($F = 9.76$, $p < 0.001$), with C showing the highest value ($16.6 \pm 1.1\%$), whereas protein content was also significantly affected by diet ($p = 0.001$), reaching the highest value under RI ($62.6 \pm 2.6\%$). The fatty acid profile was strongly influenced by dietary treatment, with significant differences detected for most individual fatty acids and aggregate fatty acid classes ($p < 0.001$). The C treatment resulted in the highest SFA and MUFA concentrations (89.6 ± 5.6 and 57.6 ± 4.0 mg g⁻¹, respectively), together with the lowest PUFA (16.4 ± 4.0 mg g⁻¹) and n-3 HUFA concentrations (5.3 ± 0.7 mg g⁻¹). In contrast, R and RI were characterized by high PUFA and n-3 HUFA concentrations, with RI reaching 61.6 ± 0.6 and 21.2 ± 0.5 mg g⁻¹, respectively. RC yielded the highest observed EPA concentration (8.0 ± 0.2 mg g⁻¹), whereas RI showed the highest DHA concentration (10.2 ± 0.1 mg g⁻¹).

Discussion

The results demonstrate that 24 h of enrichment substantially modulated the nutritional composition of *Apocyclops panamensis*, with the magnitude and direction of the response depending on the microalgal diet. The high SFA and MUFA concentrations, together with the comparatively low PUFA and n-3 HUFA levels observed under C, are consistent with a strong diet-dependent modulation of copepod lipid composition. Overall, the binary diets RI and RC provided the most favorable enrichment profiles, with RI characterized by high PUFA and n-3 HUFA concentrations and the highest DHA content, while RC yielded the highest EPA concentration. Among the monoalgal treatments, *Rhodomonas salina* showed a favorable nutritional profile, characterized by high PUFA and n-3 HUFA concentrations. Its presence in both best-performing binary combinations further suggests an important role in the nutritional enrichment of *A. panamensis*. These findings support the inclusion of *R. salina* as a key component in the formulation of binary enrichment diets and highlight the need for further investigation of its suitability as a long-term culture diet. The favorable nutritional profile observed for *A. panamensis* also supports its potential as a live feed for marine larviculture, warranting further comparison with conventional live feeds and direct evaluation in larval feeding trials.

(Continued on next page)

Acknowledgment

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ROLE OF ORGANIC ACIDS AS FEED ADDITIVES IN AQUACULTURE: HOW TO COMPOSE THE MOST EFFECTIVE BLEND

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Organic acids have been extensively studied in recent years as sustainable feed additives in aquaculture, providing a dual benefit of improving growth performance and mitigating pathogen pressure. Their antimicrobial action is primarily influenced by their ability to penetrate microbial cell membranes, a function directly related to the acid's dissociation constant (pKa).

Recent findings have shown that organic acids with pKa values between 3.1 and 5.5 demonstrate significant activity against key aquaculture pathogens such as *Vibrio parahaemolyticus*, *Aeromonas hydrophila*, *Streptococcus agalactiae* and more. The concept that the higher the pKa value the higher the antipathogenic effect of Short-chain fatty acids (SCFAs), was challenged with our interesting findings.

This pKa-centric approach supports the strategic design of feed additives that provide a foundation for antibiotic-free disease control strategies in aquaculture.

COMMUNAL CONSTRUCTION OF A FLAT OYSTER REEF FOR THE BENEFITS OF BOTH HABITAT RESTORATION AND AQUACULTURE

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Introduction

European flat oyster reefs (*Ostrea edulis*) were once abundant in European seas, but have largely disappeared due to overexploitation, decreased water quality, sedimentation, and diseases. The remaining populations are in a degraded state, hampering the provision of ecosystem functions and services (Thomas et al., 2022); habitat provision is marginal as due to low densities of oysters, the typical 3D-reef structure is lacking. Reef maintenance and growth is insufficient due to a shortage of oyster larvae and suitable settlement for oyster larvae. In regions with oyster aquaculture the lack of settled larvae limits oyster production, as farmers depend on the natural supply of larvae as starting material for aquaculture. Nature organizations, government and oyster farmers all agree that restoring an oyster reef can provide a solution. Still, there is mutual misunderstanding among stakeholders and a lack of knowledge about the successful combination of sustainable aquaculture and nature restoration.

Activities

Construction of a sublittoral flat oyster reef will take place in the Oosterschelde, the Netherlands. This former estuary and now protected natura 2000 area harbors a traditional oyster aquaculture community. Proposed activities include the selection of a suitable location and the actual construction of the reef. Monitoring of the (constructed) reef is an important part of this proposed project and will be conducted according to the BACI (Before-After, Control-Impact) approach. Monitoring will include oyster health parameters such as growth, survival, larval production and settlement, biodiversity indicators and the effect of management practices on both oyster health and biodiversity. Images collected using ROV and drop-down-camera (cf. zu Ermgassen et al., 2021) will be combined with observations by recreational divers (citizen science) and eDNA techniques for biodiversity monitoring. A management plan will be drafted in consultation with all relevant stakeholders to ensure the sustainable survival of the constructed reef. All stakeholders in the consortium will be actively involved in all steps described.

Expected long-term changes

A permanent, self-sustaining sublittoral European flat oyster reef in the Oosterschelde providing ecosystem functions and services, notably habitat and larval supply;

A durable increase in the abundance of flat oyster larvae providing a reliable supply of juvenile oysters, ultimately enhancing flat oyster production in the area;

Commitment of oyster farmers for the maintenance and protection of the European flat oyster reef;

More knowledge and acceptance of the oyster farming practices, by stakeholders and society, providing a societal 'license to produce' for farmers, and thereby promoting low-tropic aquaculture.

The project serves as an example of successful coexistence of low-tropic aquaculture and nature restoration, validated by stakeholders, and communicated to society.

Consortium

HZ UAS as project and research coordinator, UAS Leiden, 6 Dutch oyster farmers, the oyster association, 3 nature organizations, 4 governmental bodies, and an organization coordinating citizen science.

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BEYOND PROBLEM SOLVING: GENETIC PROGRESS AS A DRIVER OF EFFICIENCY AND PRODUCTIVITY

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Genetic improvement is often expected to function as a problemsolving tool, called upon to address specific challenges such as disease outbreaks, reduced survival, or declining performance. Although genetic selection can help to overcome problems (*e.g.*, Norris, 2017), this framing underestimates its more structural role in aquaculture production.

We argue that the principal value of genetic progress lies not in the resolution of individual problems, but in the sustained improvement of overall biological efficiency and productivity. Analogous patterns can be observed in other industries. In commercial aviation, for example, safety did not emerge from efforts to eliminate individual failure events, but rather from decades of cumulative, incremental improvements in aircraft design, training and operational procedures (Winter *et al.*, 2024).

A similar mechanism operates in breeding programs. Longterm genetic selection systematically improves the performance of populations, affecting multiple traits simultaneously. Genetic trends from Hendrix Genetics' commercial breeding programs for Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and whiteleg shrimp (*Litopenaeus vannamei*) demonstrate consistent improvements in growth, survival and yield. When viewed alongside broader industry performance trends, these data indicate that genetic improvement constitutes a major driver of efficiency and productivity gains in aquaculture.

By reframing genetic improvement as a longterm efficiency strategy rather than a shortterm solution provider, we seek to align expectations between breeders and producers. Recognising where genetic improvement can generate the most meaningful impact is essential to maximize its contribution to sustainable performance of the aquaculture industry.

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AGRICULTURAL SIDE-STREAMS FERMENTED WITH *Ganoderma lucidum* AS A NOVEL FUNCTIONAL FEED INGREDIENT FOR EUROPEAN PERCH *Perca fluviatilis*

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Introduction

The aquaculture sector faces increasing pressure to reduce reliance on fishmeal and soybean meal while maintaining fish performance and health. Fermentation of agricultural side-streams using fungal mycelium offers a promising strategy to enhance nutritional value and introduce functional properties (Kalaiselvan et al., 2025; Nandi et al., 2025; Yegin, 2025). This study evaluated composite mycelium meal (CMM), produced via solid-state fermentation with *Ganoderma lucidum*, as a sustainable and functional feed ingredient for European perch (*Perca fluviatilis*).

Materials and Methods

Juvenile perch (initial body weight 35.0 g) were fed four isonitrogenous, isolipidic, and isoenergetic diets in which CMM was progressively included at 0%, 5%, 7.5%, and 10% while maintaining diets isonitrogenous, isolipidic, and isoenergetic through balanced reformulation. These inclusion levels corresponded to soybean meal replacement levels of 0%, 25%, 37.5%, 50%, respectively. The CMM was manufactured from agricultural side-streams identified in prior preference trials, consisting of 80% canola meal, 15% sunflower meal, and 5% sunflower hulls. Diets were tested in triplicate, and the growth trial was conducted in a recirculating aquaculture system over 84 days. Growth performance, nutrient digestibility, organosomatic indices, histology, blood biochemistry, immune parameters, and gut microbiome composition were assessed.

Results and Discussion

CMM inclusion up to 10% did not negatively affect growth performance, feed conversion ratio, or survival. Fish fed higher inclusion levels (7.5–10%) exhibited significantly ($P < 0.05$) increased body weight (85.0 g and 93.2 g ± 15 respectively) compared to controls (78.5 g ± 15.1). Nutrient digestibility remained stable across all treatments, indicating efficient utilization of the fermented ingredient.

Organosomatic indices and histological assessments showed no adverse effects, suggesting maintained physiological condition. Blood biochemistry revealed limited diet-related changes, although cholesterol and alkaline phosphatase varied with CMM inclusion.

Immunological analyses demonstrated clear functional benefits: hepatic lipid peroxidation decreased in a dose-dependent manner, while intestinal lysozyme activity and skin mucus protein content increased, indicating enhanced oxidative status and innate immune response.

Gut microbiome analysis revealed diet-dependent shifts in community composition, with reduced diversity but increased specialization at higher inclusion levels, suggesting interactions between diet and microbial ecology.

Conclusion

Fermented agricultural side-streams fermented with *G. lucidum* mycelium can replace soybean meal in European perch diets up to 50% without compromising growth or health. Additionally, the ingredient provides functional benefits related to immune response and oxidative stress mitigation. This approach represents a promising strategy for improving sustainability and functionality in aquaculture feeds.

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Acknowledgment

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BALANCING TEMPERATURE AND SALINITY TO IMPROVE GROWTH AND SURVIVAL OF *Ruditapes decussatus*

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Introduction

Larval development is one of the most critical stages in bivalve hatchery production, with environmental conditions strongly influencing growth, survival and seed quality. Temperature and salinity are among the main drivers of larval physiological performance, yet they are commonly investigated independently despite acting simultaneously in both natural and hatchery environments. Understanding their combined effects is essential because their interactions may generate biological responses that cannot be predicted from single-factor studies. Although, the grooved carpet shell (*Ruditapes decussatus*) is a high-value species in southern Europe, information on the interactive effects of temperature and salinity during larval development remains scarce. This study evaluates how the combined influence of these two environmental factors affects larval viability, providing practical information to optimize commercial hatchery production.

Materials and Methods

Ruditapes decussatus larvae at 5 days post-fertilization (dpf) were randomly distributed among eighteen 5 L-beakers (triplicate per treatment). Larval density was adjusted throughout the experiment with 5, 3 and 1.5 larvae ml⁻¹ at 5, 14 and 17 dpf, respectively. Larvae were maintained under two temperatures (18 and 22 °C) and three salinities (25, 34 and 36) until 19 dpf. Water was renewed every two days with filtered and UV-sterilized seawater and continuous aeration. Larvae were fed with 100 cells µl⁻¹ of a bispecific microalgae diet, consisting of 60% *Tisochrysis lutea* and 40% *Chaetoceros calcitrans* until 17 dpf, followed by 40% *T. lutea* and 60% *C. calcitrans* from 18 dpf onwards. Every two days, the proportion of live larvae (%) was calculated as the number of live larvae divided by the total number of live and dead larvae counted, and shell growth was assessed by measuring shell length from digital images using ImageJ®. Data and statistical analysis were performed using R version 4.5.3.

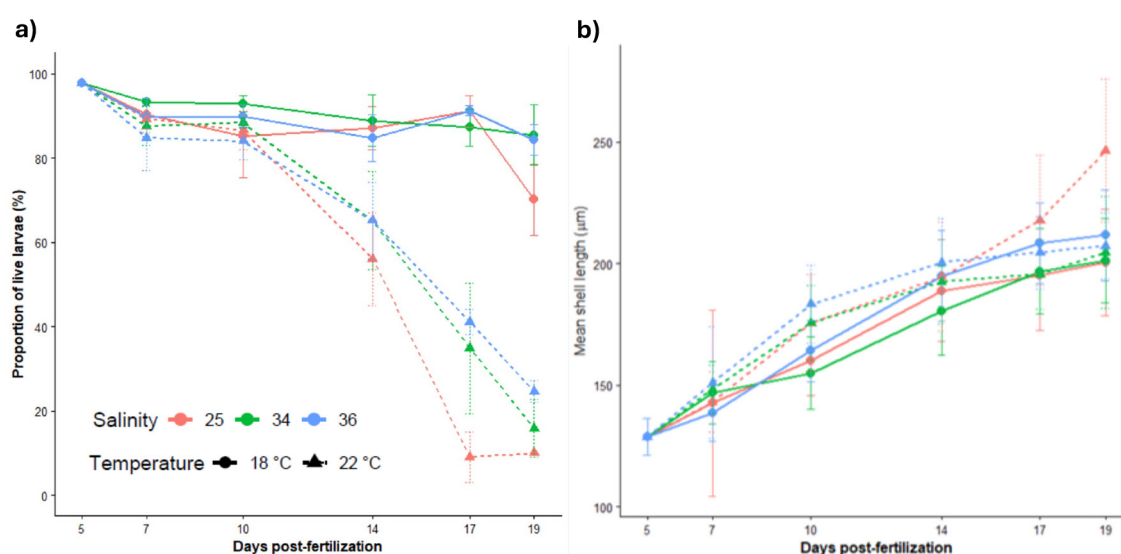


Figure 1. (a) Mean of the proportion of live *Ruditapes decussatus* larvae (%), and (b) mean shell length (µm) over the experimental period under different temperature and salinity conditions. Data were expressed as mean ± SD (n=3).

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Results and Discussion

The proportion of live larvae remained high (> 85%) and did not differ significantly among treatments during the first five experimental days, likely reflecting the mixotrophic phase (Matias *et al.*, 2011), during which endogenous energy reserves may partially buffer the effects of temperature and salinity. From 9 dpf onwards, temperature became the main factor affecting the proportion of live larvae ($P < 0.001$). By the end of the experiment, the proportion of live larvae remained above 70% at 18 °C, whereas it decreased to below 30% at 22 °C, particularly under low salinity. This reduction at 22°C may be associated with increased metabolic demands and enhanced bacterial proliferation under warmer conditions (Dubert *et al.*, 2017). Shell length increased progressively throughout larval development under all experimental conditions, with no statistically significant differences among treatments. However, larvae reared at 22 °C consistently showed a tendency towards greater shell growth than those maintained at 18 °C. This enhanced growth was accompanied by a lower proportion of live larvae, suggesting a trade-off between growth and larval performance under warmer conditions. These findings highlight the importance of evaluating temperature and salinity simultaneously, as their combined effects should be considered when defining optimal hatchery rearing conditions for *Ruditapes decussatus*.

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INTERPLAY BETWEEN DISINFECTION AND THE ENIGMATIC DIPLOMONAD PARASITE *Spironucleus Salmonicida* IN ATLANTIC SALMON

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Introduction

The diplomonad parasite *Spironucleus salmonicida* is the causative agent of spironucleosis, a severe systemic disease in Atlantic salmon characterised by haemorrhage, organ enlargement, and granulomatous lesions. Although outbreaks are sporadic, their impact can be devastating, and no approved treatment is currently available, making prevention through biosecurity the primary control strategy. In land-based aquaculture, especially recirculating aquaculture systems (RAS), water disinfection represents a critical barrier against pathogen introduction and spread. Ultraviolet (UV) irradiation and ozonation are widely applied disinfection technologies, yet limited data exist regarding their effectiveness against *S. salmonicida* or their broader effects on fish health and the microbial ecology of RAS. This study investigated the susceptibility of *S. salmonicida* to UV irradiation under controlled conditions and assessed the outcomes of different RAS loop-disinfection strategies following a simulated biosecurity breach involving Atlantic salmon smolts.

Materials and Methods

The study combined *in situ* UV exposure experiments with an *in vivo* RAS challenge trial. For the *in situ* experiments, cultured *S. salmonicida* trophozoites isolated from diseased Atlantic salmon were exposed to increasing doses of low-pressure (LP) and medium-pressure (MP) UV irradiation under controlled brackish water conditions. Parasite viability was assessed through motility observations and vital staining, with follow-up incubation to evaluate regrowth.

The *in vivo* trial was conducted using nine independent experimental RAS units stocked with Atlantic salmon smolts. Three treatments were applied in triplicate: no RAS loop disinfection (control), continuous LP UV disinfection, and continuous ozone treatment. After a two-week acclimation period, a simulated biosecurity breach was performed by introducing cultured *S. salmonicida* into the make-up water for three consecutive days. Fish were monitored for four weeks post-breach. Growth performance, gross pathology, and infection status were evaluated. Parasite presence was assessed using RT-qPCR across multiple tissues, peritoneal fluid, tank walls, and biofilter media. Gill, skin, and gut tissues were examined histologically and transcriptionally to assess mucosal integrity, inflammatory responses, and oxidative stress. Additionally, microbial communities on tank walls and biofilter media were characterised using full-length 16S rRNA gene sequencing.

Results

In situ, MP UV irradiation rapidly inactivated *S. salmonicida*, achieving ≥ 5 -log reductions in viability at doses of 50 mJ/cm² and above, with no evidence of regrowth. LP UV irradiation was less effective, requiring substantially higher doses for comparable reductions, and parasite recovery was observed at lower doses.

In the *in vivo* RAS trial, no fish developed clinical spironucleosis, and *S. salmonicida* was not detected in any tissue, environmental sample, or culture attempts, irrespective of disinfection treatment. Growth performance and condition factor did not differ among treatments. Histological evaluation showed no treatment-related impairment of gill or skin mucosal integrity. However, ozone treatment induced a pronounced upregulation of oxidative stress-related genes in the gills and skin, while UV treatment had minimal molecular impact. Water quality was improved by both disinfection strategies, with higher UV transmittance and lower turbidity compared to the control.

Microbial analyses revealed distinct communities between biofilter media and tank walls, with disinfection exerting selective pressures on community composition. UV and ozone treatments promoted different microbial taxa, although overall microbial diversity remained stable across treatments.

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Discussion

The findings demonstrate that *S. salmonicida* is highly sensitive to MP UV irradiation under controlled conditions, supporting the potential of UV-based disinfection as a biosecurity measure in salmon RAS. The failure of the simulated waterborne biosecurity breach to establish infection suggests that transmission dynamics are more complex than previously assumed and may involve additional stressors or vectors. While both UV and ozone improved water quality without compromising fish growth or mucosal integrity, ozone elicited measurable oxidative stress responses, indicating the need for careful operational control. Overall, UV disinfection appears to offer an effective and biologically mild approach for strengthening RAS biosecurity against *S. salmonicida*, while highlighting the importance of considering host, pathogen, and environmental interactions.

Acknowledgement

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RASRACE FOR NEXT-GENERATION RECIRCULATING AQUACULTURE: MODULAR CONTAINMENT AND SCALING OUT TO REDUCE COMPLEXITY, RISK AND COST

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Background and problem statement

Large land-based recirculating aquaculture is expanding quickly, but many flagship facilities still combine high capex and energy use with low tolerance to process upsets. Recent high-profile projects—and failures—have largely involved salmon, yet the core lessons are architectural and transferable across freshwater and seawater farming (finfish, crustaceans, aquaponics and other multitrophic co-productions). A dominant “plant-first” logic scales biomass around a fixed, centralised treatment plant: it can lower unit costs on paper, but it also couples biological and technical risk, magnifies single-point failures (power, pumping, water chemistry), and drives operational and compliance complexity. Meanwhile, sustainability assessment is shifting from within-farm discharge metrics toward regional nutrient carrying capacity and valorisation pathways.

Key challenges in state-of-the-art RAS (salmon as reference case)

In large grow-out RAS (salmon often the reference case), four challenge clusters dominate: (i) capex concentrated in central life-support and treatment, incentivising ever larger volumes to dilute fixed costs; (ii) opex exposure from pumping head, temperature control, oxygen and CO₂ management, buffering chemistry, and energy-price volatility; (iii) systemic risk from tightly coupled loops and limited buffering, where disturbances can escalate across large biomasses; and (iv) increasing assurance, monitoring, and reporting requirements that translate into more unit processes, sensors, automation, and procedures. The common response is “technology stacking” to treat symptoms; we argue for architectural simplification instead.

The RasRace concept: designing for simplicity, modularity and containment

RASRace is a “module-first” alternative to plant-first RAS. Each production module is self-contained (tank + essential treatment and control) rather than dependent on a single, central plant. The concept uses ring-shaped tanks (“raceway”) to create controlled hydrodynamics and short, manageable residence times, with physical access designed for rapid inspection, cleaning, and maintenance. Decentralising critical functions compartmentalises biological risk, reduces single-point failures, and supports stepwise capacity addition. Economically, standardised modules improve repeatability and learning rates—shifting investment logic from scaling up one bespoke facility to scaling out replicable contained units.

From effluent control to regional nutrient balancing

Beyond within-farm effluent control, circular nutrient outcomes are often constrained by regional nutrient-handling capacity and logistics. High-retention systems can concentrate sludge and dissolved nutrients, but benefits depend on practical routes for stabilisation, transport, and valorisation (e.g., fertiliser, biogas, or as growth media for heterotrophic microorganisms). We therefore propose judging recirculating systems by how well they enable simple, auditable nutrient capture and routing—not only by internal water quality and unit cost. In parallel, the CircularRainbow project (Interreg Italia-Slovenija) has investigated RAS for Mediterranean contexts where summer water scarcity can restrict flow-through land-based aquaculture. Such typology comparisons enable sustainability assessments across aquaculture typologies. Overall, the presentation highlights design choices that prioritise containment, compartmentalisation, and nutrient balancing—supporting scaling out modular units (rather than scaling up single plants) and reducing reliance on add-on complexity.

OPTIMIZING *Artemia* HARVEST TIMING/ BALANCING BIOMASS YIELD AND NUTRITIONAL QUALITY DURING EARLY INCUBATION

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Introduction

The first 36 hours of incubation of dried *Artemia* cysts represent a metabolically intensive phase of development, during which embryonic growth is entirely supported by nutrient reserves stored within the yolk sac. As development progresses through early instar stages, these internal reserves are gradually depleted, directly influencing the nutritional value of the resulting live feed.

For fish and shrimp hatcheries, *Artemia* production is often approached with a focus on harvesting specific developmental stages, such as early Instar I nauplii, due to their perceived higher nutritional quality. However, this approach may overlook the broader production dynamics. Extending incubation time allows a greater proportion of cysts to hatch and develop into a harvestable population, increasing overall biomass output. Rather than targeting a single developmental stage, hatchery efficiency can benefit from an optimized balance between total biomass production and preservation of nutritional quality. This implies managing incubation conditions and harvest timing to maximize overall yield while minimizing depletion of endogenous reserves. In this context, harvesting a mixed population of Instar I and II nauplii can represent a practical and efficient strategy, provided that system conditions are optimized to ensure high output and controlled nutritional decline.

The objective of this study is therefore to evaluate the evolution of overall nutritional composition, average nauplius dry weight (IDW), and total nauplius biomass (IWW) during the first 36 hours of *Artemia* incubation, with a focus on defining optimal production windows that maximize total feed availability while maintaining adequate nutritional value.

Materials and methods

EG SEP-Art D-FENSE™ cysts sourced from the Great Salt Lake (Utah, USA) were incubated under standardized hatching conditions (25 ppt salinity, 29 °C, pH 8.25). Samples were collected periodically over 36 h of incubation in six replicate hatching tanks to assess hatching kinetics, individual dry weight (IDW), individual wet weight (IWW), fatty acid composition, and proximate nutritional profile.

Samples were taken for each hatching tank replicate. The cyst shells were removed with a magnet. The nauplii were collected on a 118µm sieve and rinsed with RO water. A weighed portion of the nauplii were then reintroduced to a 1L Imhoff cone, which was filled to 1L with RO water. 250µL of sample from each cone replicate was pipetted into each well of a 3x4 cell culture well plate, with one plate sampled for each replicate. Each well was then treated with a fixing solution and counted using the SnappArt L-SENSE. Results from the SnappArt portal were averaged for each replicate of the given sampling time. Once the well plate samples were taken, 100mL samples from each replicate were taken and collected on pre-weighed dried 118µm sieves in triplicate. The sieves were then dried at 60°C for 24h and weighed to determine the total dry weight of the animals. The IDW and IWW were then calculated and averaged according to replicate.

The remaining nauplii were gathered and analyzed using fatty acid methyl ester (FAME) analysis, and proximate composition was measured using standard nutritional assays.

Results

Results showed a significant increase in individual wet weight (IWW) from 10.99 µg nauplius⁻¹ at 20 h to 17.74 µg nauplius⁻¹ at 36 h of incubation. In contrast, individual dry weight (IDW) declined over the same period, decreasing from 2.18 µg nauplius⁻¹ at 20 h to 1.90 µg nauplius⁻¹ at 36 h. This reduction in IDW was primarily driven by losses in carbohydrates, which declined from 0.5994 to 0.3719 µg nauplius⁻¹, and fatty acids, which decreased from 0.3853 to 0.2808 µg nauplius⁻¹.

A notable decline in IDW occurred during the transition from the freshly hatched umbrella stage to instar I, while smaller losses were observed during development from instar I to instar II. The IDW loss was the largest between 30 h and 36 h of incubation. Fatty acid utilization was selective, with polyunsaturated fatty acids exhibiting the greatest reductions over the hatching period, particularly 18:3(n-3) (α-linolenic acid). Among monounsaturated and saturated fatty acids, 18:1(n-9) (oleic acid) and 16:0 (palmitic acid) showed the largest decreases respectively.

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Discussion

These findings suggest that early *Artemia* harvesting (18–21 h) may not always represent the optimal strategy for commercial hatcheries. While instar I nauplii are often considered nutritionally superior to later stages, this advantage may be outweighed by increased nauplius availability under longer incubation.

Importantly, the commonly held view that Instar II nauplii are inherently inferior to Instar I should not be interpreted as a strict or binary distinction. In practice, it is often assumed that all *Artemia* hatched beyond ~22 hours (e.g., 22–48 h) represent a uniform Instar II population with similar nutritional characteristics. However, this work clearly demonstrates that early Instar II nauplii differ significantly from later developmental stages that are often broadly, and sometimes incorrectly, grouped under the same label.

Specifically, early Instar II nauplii remain nutritionally very close to late Instar I, with only limited depletion of internal reserves. In contrast, at extended incubation times (>30 h), the population increasingly consists of more advanced Instar II and even later stages, where metabolic activity has led to a substantial consumption of endogenous reserves. It is within this later phase, rather than the transition from Instar I to early Instar II, that the most pronounced decline in nutritional quality occurs.

At these later stages, where reserve depletion becomes more pronounced, enrichment strategies should be considered to compensate for nutritional losses and optimize the feed quality. Therefore, rather than focusing strictly on stage-specific harvesting, optimizing *Artemia* production should aim at balancing total biomass output with controlled nutrient depletion, allowing the efficient use of mixed-stage populations while maintaining overall nutritional performance.

ALGAL TOXINS IN FISH FEED AND ITS IMPACT ON ATLANTIC SALMON

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Introduction

As the aquaculture industry moves toward more sustainable feed solutions, low-trophic marine organisms such as mussels and tunicates are gaining increasing attention as alternative feed ingredients. Their favorable protein and fatty acid profiles make them attractive substitutes for conventional resources such as fish meal and fish oil¹. However, as filter feeders, these organisms can bioaccumulate algal toxins during harmful algal blooms. Toxins such as okadaic acid and dinophysistoxins (OA/DTXs), as well as saxitoxins (STX, NEO, GTXs, and C-toxins), may persist in their tissues² and could be transferred to fish when incorporated into feed, posing potential risks to fish health and food safety. Therefore, understanding the bioaccumulation and transfer of these toxins is essential and forms the basis of the present study.

Materials and Methods

This study evaluates the potential impact and transfer of selected algal toxins in salmon feed through a controlled, stepwise feeding trial using Atlantic salmon (*Salmo salar*) parr and post-smolt. Diets were formulated using commercial ingredients and top-coated with purified OA/DTXs solubilised in ethanol, or saxitoxins solubilized in water. Fish feed was formulated with three levels of each toxins for parr trial and two levels for post smolts. Fish were fed with experimental diets until they doubled in body weight. Feed intake, growth performance, and behavioural responses were monitored throughout the trial. Samples especially, from water, leftover feed and fish were collected for toxin quantification using LC-MS methods; and tissue samples for gene expression analyses.

Results and Discussion

In salmon parr, specific growth rate and thermal growth coefficient were slightly reduced in the high-dose saxitoxin group compared to the control, whereas no effects on growth were observed in fish fed okadaic acid group (OA/DTXs). In post-smolt, no significant effects on growth performance or welfare were observed for any of the toxin treatments. No abnormal behaviour or signs of stress were recorded in either trial.

LC-MS analysis showed elevated toxin levels in the intestine and low levels in the liver at the highest exposure levels in parr. No accumulation of toxins was detected in fillet samples. Post-smolt analyses are ongoing.

Microarray analysis revealed no significant changes in liver gene expression in parr exposed to STX or OAs. However, in post-smolts, OA exposure was associated with differential expression of genes related to lipid metabolism, xenobiotic processing, redox signalling, and cellular functions in the intestine and liver. Whereas, STX exposure resulted in minimal changes in gene expression across tissues.

The maximum dietary inclusion levels were based on 95% of the highest reported toxin concentrations in Norwegian shellfish, assuming a 5% inclusion of shellfish in feed. These findings contribute to the risk assessment of algal toxins in emerging marine feed resources for salmon.

Acknowledgment

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EFFECTS OF PROTEIN HYDROLYSATES ON GILTHEAD SEABREAM *Sparus aurata* JUVENILES IMMUNE STATUS

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Introduction

Early-life stages are among the most sensitive periods in fish development, during which exposure to environmental, husbandry, and nutritional stressors can impair welfare, growth performance, physiological resilience, and survival (Ramos et al., 2021). Therefore, inadequate nutrition during early development can result in significant mortality and economic costs. Bioactive peptides are typically composed of 2–20 amino acids, and are widely recognized for their diverse biological functions. The incorporation of bioactive peptides into aquafeeds is increasingly regarded as an effective nutritional strategy to enhance the resilience of farmed aquatic species against stress-induced damage and pathogen infections (Chen et al., 2025). In fish, specific peptide fractions have been associated with improved antioxidant capacity, regulation of innate immune responses, support of intestinal function, and enhanced tolerance to environmental and husbandry-related stressors. However, the effects of bioactive peptides during early developmental stages in fish remain insufficiently characterized. The present study aimed to evaluate the effects of a diet fortified with bioactive peptides derived from poultry feathers and whey protein hydrolysates on the intestinal innate immune response of early juvenile gilthead seabream (*Sparus aurata*).

Materials and Methods

Gilthead seabream juveniles (5.8 ± 0.4 cm total length; 2.4 ± 0.5 g weight) were randomly distributed into experimental tanks at an initial stocking density of approximately 1,000 fish per tank. Two dietary treatments, each in duplicate, were evaluated: a commercial aquafeed (CTRL) and an experimental functional diet (FOR) fortified with bioactive peptides derived from poultry feather hydrolysate and whey protein hydrolysates. Fish were hand-fed to satiety three times per day. Gut samples were collected in eight fish per tank at 7, 14, and 45 days after feeding, snap-frozen in liquid nitrogen, and stored at -80 °C until processing. Nitric oxide levels as well as bactericidal and peroxidase activities were analysed following gut samples homogenization. Data was tested for normality and homogeneity of variances using Shapiro–Wilk and Levene’s tests, respectively. Data was analyzed using the JAMovi software (version 2.7.38), using the tank as the experimental unit. Data was plotted in GraphPad Prism 7 for Windows. The Independent Samples t-test and Mann-Whitney U-test were used to assess differences between groups. A repeated measures ANOVA or the Friedman Test was used to compare values between days within groups, considering sampling day as between-subjects factor and immune activity as within-subjects factor. Statistical significance was accepted at $p < 0.05$ for all comparisons.

Results

Gut nitric oxide levels decreased in gilthead seabream juveniles fed the FOR dietary treatment compared to their counterparts fed CTRL after 14 days of feeding. Moreover, intestinal bactericidal activity showed a similar trend with lower levels observed in seabream fed the FOR dietary treatment than those fed CTRL after 7 days of feeding. A time effect was also observed in the intestine of seabream juveniles fed CTRL, with higher peroxidase and bactericidal activities recorded after 7 days of feeding than those levels observed at 14 and 45 days, respectively. In contrast, no time effect was observed in seabream fed the FOR dietary treatment throughout the feeding trial.

Discussion

The CTRL diet, based on a commercial formulation, appeared to induce stronger innate immune activation in seabream juveniles than the FOR dietary treatment, particularly after a short-term feeding period. In contrast, the FOR dietary treatment elicited milder immune modulation, suggesting that the inclusion of poultry feather and whey protein hydrolysates in diets for early juvenile gilthead seabream may require further refinement to improve mucosal immune status. Therefore, further studies are needed to better understand the immunomodulatory potential of protein hydrolysates during the early life stages of gilthead seabream.

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OPTIMIZING LOW-SALINITY ACCLIMATION PROTOCOLS IN THE GREY MULLET (*Chelon labrosus*): OSMOREGULATORY AND LIPID METABOLISM RESPONSES

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Introduction

Mulletts are catadromous teleost that migrate to coastal environments during their juvenile stage (Whitfield & Patrick, 2015) and are considered promising candidates for sustainable aquaculture within the context of blue growth due to their omnivorous feeding habits and tolerance to a wide range of salinities (Krause *et al.*, 2022). Among these species, the thick-lipped grey mullet, *Chelon labrosus*, has demonstrated a remarkable ability to convert 18-C fatty acids into long-chain polyunsaturated fatty acids (LC-PUFAs), particularly under near-isoosmotic conditions (~16 ppt) (Quirós-Pozo *et al.*, 2025). However, despite its tolerance to low salinities, optimal growth has been reported at intermediate salinities (10–16 ppt). Reduced survival has been observed at lower salinities, indicating that salinity plays a key role in its physiological performance and highlights the need to better understand fish responses to acclimation and physiological limits as salinity management could therefore enhance fish welfare, lipid metabolism, and the overall efficiency of aquaculture systems. The aim of the present work was to understand the effects of different salinity gradients (36 to 0 ppt) on *Chelon labrosus*.

Materials and Methods

A total of 126 juveniles of *Chelon labrosus* were distributed across nine 500 L tanks, within a recirculating aquaculture system equipped with independent mechanical and biological filtration units. Three experimental treatments were established (triplicate tanks each): (C) control (constant 36 ppt), (S2) fast gradient salinity reduction (36 to 0 ppt over 18 days), and (S3) slow gradient salinity reduction (36 to 0 ppt over 24 days). Water quality parameters were monitored daily, as sampling was conducted at key salinity points according to review (36, 16, 5, and 0 ppt) throughout the one-month trial. At each sampling point, fish were collected and blood samples were obtained in heparinized tubes, centrifuged to isolate plasma for the analysis of cortisol, glucose, lactate, and triglycerides using commercial assay kits. Data was analyzed using ANOVA.

Results

No mortality was observed under any of the applied salinity gradients throughout the experimental period. Plasma cortisol levels in *Chelon labrosus* were significantly affected by salinity and acclimation rate. At 16 ppt, both fast (S2, 7days) and slow (S3, 14days) acclimation treatments exhibited significantly higher cortisol levels compared to the control (36 ppt), indicating an acute stress response associated with salinity reduction. At 5 ppt, significant differences were detected among treatments (Tukey's post hoc test, $p < 0.05$), with fish under fast acclimation (S2) showing significantly higher cortisol levels than both the control (C) and slow acclimation (S3) treatments, which displayed similar values. At the final salinity (0 ppt), cortisol levels decreased across treatments, with the lowest values recorded in the slow gradient treatment. Cortisol levels exhibited a treatment-dependent pattern, with an initial increase and a subsequent decline at the end (Figure 1). Regarding metabolic responses, plasma lactate levels at 16 ppt showed significant differences among treatments (Tukey's post hoc test, $p < 0.05$), with higher values observed in S2 compared to the control, while S3 displayed intermediate values. Fatty acids (FAMES) and lipid profile are currently being processed to further evaluate metabolic adjustments associated with salinity acclimation.

Discussion

The increase in plasma cortisol at intermediate salinity (16 ppt) confirms that salinity reduction induces a physiological stress response in *Chelon labrosus*, consistent with previous reports on euryhaline teleosts exposed to osmotic fluctuations. Differences between acclimation strategies indicate that the rate of salinity change is a key factor modulating endocrine stress responses. Fast gradient (S2) resulted in sustained cortisol elevation at low salinity, whereas slow gradient (S3) promoted a faster return toward baseline levels. These findings are consistent with the concept of stress preconditioning, whereby exposure to sublethal environmental change can enhance physiological resilience to subsequent abiotic stress through conserved cellular stress response mechanisms in fish (Evans & Kültz, 2020; Quirós-Pozo *et al.*, 2025). Overall, the results support the importance of controlled acclimation strategies to optimise physiological stability and welfare in *Chelon labrosus* under low-salinity aquaculture conditions.

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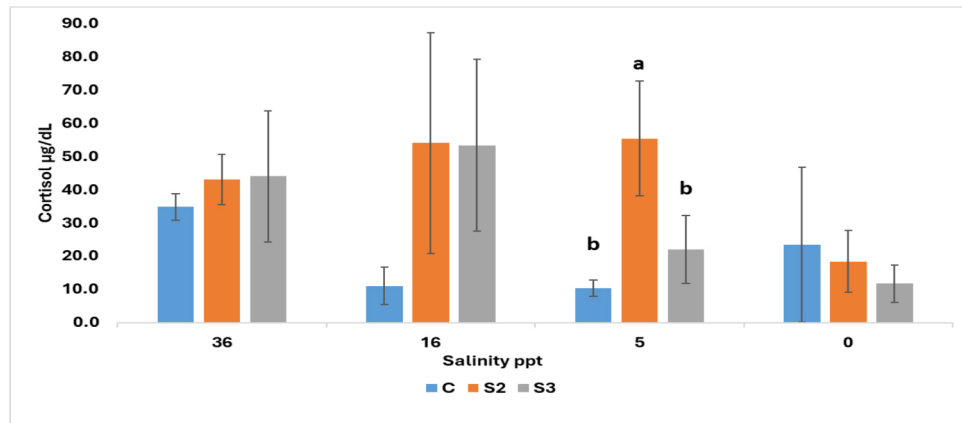


Figure 1. Effect of salinity acclimation rate on plasma cortisol ($\mu\text{g/dL}$) in *Chelon labrosus*, in all the treatments C (Control), S2 (Fast) and S3 (Slow) and the salinities 36, 16, 5 and 0 ppt.

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RARE EARTH ELEMENT-CONTAINING NANOPARTICLES IN FRESHWATER SYSTEMS: RESPONSES ACROSS TROPHIC LEVELS

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The increasing use of rare earth element (REE)-containing nanoparticles in biomedical, imaging, and sensing technologies may contribute to their release into surface waters. Their environmental transformation and biological effects may influence water quality, primary productivity, natural food availability, and fish health. However, their potential consequences for freshwater systems remain insufficiently understood.

This study evaluated the biological effects of two types of REE-containing nanoparticles across three freshwater trophic levels. The green alga *Desmodesmus communis* was exposed for 96 h to 0–100 $\mu\text{g mL}^{-1}$ $\text{NaYF}_4\text{:Yb,Er}$ upconverting nanoparticles in modified Wilkins–Chalgren medium and Neris River water. *Daphnia magna* and zebrafish (*Danio rerio*) embryos and larvae were exposed to Eu^{3+} -doped GdPO_4 nanoparticles at concentrations up to 100 $\mu\text{g mL}^{-1}$. Algal growth and physiological responses, *D. magna* heart rate and swimming behaviour, and zebrafish survival, hatching, cardiac activity, and early-life behaviour were assessed.

REE-containing nanoparticles predominantly induced sublethal and organism-specific responses. In *D. communis*, multivariate analysis revealed concentration-dependent changes in the overall response profile, particularly at higher nanoparticle concentrations. However, biological responses were more strongly influenced by the exposure medium than by nanoparticle concentration. In *D. magna*, cardiac responses varied among nanoparticle morphologies, while swimming distance and velocity generally decreased with increasing concentration. In zebrafish, embryo burst activity increased at higher concentrations. Larval heart rate was stimulated at intermediate concentrations but returned close to the control level at the highest concentration.

These findings demonstrate that REE-containing nanoparticles can affect interconnected components of freshwater food webs relevant to freshwater systems. Alterations in primary producers and zooplankton may influence natural food availability and trophic transfer, whereas physiological and behavioural changes in fish early life stages may affect larval performance. Nanoparticle morphology, environmental transformations, and realistic water conditions should therefore be considered when assessing emerging contaminants in aquatic ecosystems.

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CIRCULARITY OF WASTE IN RAS AQUACULTURE – THE USE OF BY-PRODUCTS FOR TILAPIA FEED

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Alternative feed resources from circular economy

Renewable resources and their efficient utilisation are key to make aquaculture production as sustainable and profitable as possible. The UrbanAqua project conceptualises a circular economy in which residual materials and by-products are utilised directly and nutrients are upcycled within the loop. Three raw materials were identified for excellent residue and nutrient conversion: Duckweed (*Lemna minor*), insects (larvae of *Hermetia illucens* and legs of *Acheta domesticus*) and the cyanobacterium spirulina (*Arthrospira platensis*), all of which are also suitable and promising candidates for use in aquafeeds (Eide et al., 2024, Soma et al., 2024, Michini and Michael, 2024). While recent research clearly shows use of plant-based products in the fish feeds can lead to a reduction in growth performance due to antinutritional factors, these negative effects can be minimized to a negligible level through carefully designed ingredient combinations (Turchini et al., 2019).

Such holistic feed approaches can provide a sustainable and regionally producible feed solution for land-based aquaculture and create new value chains. These developments align with emerging approaches that integrate microbially driven nutrient recycling with engineered systems converting agricultural waste streams into value-added biomass (Glockow et al., 2024).

The research work carried out here not only demonstrates the potential of the simple combination of the above-mentioned feed raw materials compared to a commercial formulation, but also provides insights into the effects of the respective sustainable feed raw materials duckweed, insects and spirulina on growth performance, metabolic and morphologic parameters. A total of six different diets were designed. A control diet was formulated to reflect a standard commercial composition. Diet 2 was designed following the commercial control; however, the test ingredients were incorporated as the principal raw materials, while fishmeal and fish oil remained included. Diet 3 was formulated entirely without fishmeal and fish oil, substituting these components with alternative sustainable ingredient sources. Diets 4–6 represented experimental formulations in which the test ingredients were incorporated at increasing inclusion levels (table 1).

Research Finding

Preliminary results of the 60-day trial show that the weight gain of fish that received the sustainable feed ingredients from circular economy was significantly increased compared to the conventional formulation ($p < 0.002$). The increased use of insect meal led to compensation of growth depression caused by the omission of fish meal and fish oil in the sustainable test diets. The analysis of plasma levels of glucose, triglycerides, cholesterol, and total protein supports the findings from the growth parameters, allowing for the creation of a detailed overall picture. The feed formulation using duckweed, insect meal, and spirulina as a replacement — with minimal inclusion of fishmeal and fish oil showed optimal blood parameters, liver values, and growth. These results were similar for the feed that was free of fish meal and fish oil and an above-average amount of insect meal. The only difference observed was a slight increase in blood lipid levels, indicating potential for further optimization of the feed, although growth rates were high ($\text{SGR} = 2.3 \text{ \% bodyweight day}^{-1}$).

The study shows the suitability of different by-products that have arisen from residue streams from the recirculation of different species. However, further optimisation steps are necessary with regard to the formulation. Sustainability aspects such as nutrient recovery and by-product inclusion can be used to pioneer production of fresh fish products in urban areas.

(Continued on next page)

Table

Diet	Description	Fish meal and oil	Sustainable alternative oils
Commercial Control	Commercial formulation	✓	
Test Control	Closely after the commercial control; however, the test ingredients were incorporated as the principal raw materials.	✓	
Sustainable Control	Closely after the test control. Entirely without fishmeal and fish oil, with alternative and more sustainable ingredient sources		✓
Increased Spirulina	Closely after the sustainable control but with increased amounts of Spirulina		✓
Increased Insects	Closely after the sustainable control but with increased amounts of insect meal		✓
Increase Waterlentils	Closely after the sustainable control but with increased amounts of waterlentils		✓

*Table 1. Summary of experimental diets***Acknowledgment**

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DIETARY MICROBIAL OMEGA-3 OILS ENHANCE FATTY ACID PROFILE OF RAINBOW TROUT FILLETS WITHOUT DISRUPTING GUT HEALTH

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Introduction

This study was conducted within a broader project aimed at improving the nutritional quality of marine and freshwater fish fillets by enhancing nutrient transfer from raw materials to the final product. The ultimate goal is to deliver fish products with optimal nutritional value that meet consumer expectations.

Omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs), especially eicosapentaenoic and docosahexaenoic fatty acids (EPA and DHA), are essential nutrients in the human diet and are mainly obtained from the consumption of fish products. In the present study, a microbial oil rich in EPA and DHA was investigated as a feed additive for rainbow trout (*Oncorhynchus mykiss*). In addition to assessing the accumulation of these fatty acids in the fillet, we evaluated their effects on fish physiology and health.

Methods

Two different inclusion levels of microbial oil were investigated in a 90-day feeding trial. At the beginning of the trial, 810 juvenile rainbow trout were randomly distributed into nine 500 L fiberglass tanks. Each tank was randomly assigned to either the control diet or one of the microbial oil diets (3 tanks per group). At the end of the feeding trial, fillet samples were collected to assess their fatty acid profile. Gas chromatography coupled with mass spectrometry was employed for the purpose. Moreover, the composition and functionality of the gut microbiota were analyzed using high-throughput sequencing techniques previously described in publications by our group (Hasan et al., 2024; Rimoldi et al., 2025; Kalemi et al., 2025).

Results

Dietary microbial oil at higher inclusion levels led to an enrichment of n-3 LC-PUFAs, especially EPA and DHA, in the fillets of rainbow trout. Consistent with this increase, the ratio of omega-3 to omega-6 PUFAs was higher in the rainbow trout fillets fed the experimental diets. Overall, the fatty acid profile in the fillets matched that of the diets, as previously observed in a similar study (Terova et al., 2021). As for the gut microbiota status, subtle qualitative shifts were detected at the highest inclusion level, but the overall microbial diversity, dominant taxa and predicted functional pathways remained largely unchanged between the feeding groups.

Conclusions

This study highlights the importance of aquafeed not only for fish growth and health, but also for the nutritional quality of the final product. In particular, it emphasizes the role of feed formulations in shaping the nutritional quality of farmed fish for human consumption. Our results showed that the addition of microbial oil to rainbow trout feed increases the content of omega-3 PUFAs in the fillet 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 fillet. Understanding how different nutritional strategies affect gut microbiota, metabolic outcomes and fillet composition is essential for the development of more sustainable and nutritionally valuable aquaculture products.

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Acknowledgments

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FARMING MUSSELS (*Mytilus edulis*) IN A DUTCH OFF-SHORE WIND FARM

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The Borssele windfarm is situated 24 km off shore the coast in the Southwest of The Netherlands. The government designated several areas for multi use: mariculture, passive fishing, nature development and other types of renewable energy. OOS SMF developed a Semi-Submersible Mussel Farm (SMF). The test version of the SMF is located in the Borssele windfarm. It consists of a 10 x 10 x 10 m platform (moored stationary) that functions as the anchoring point on one side for 5 longlines of 200 m while the other end of the longlines is fixed to buoys. In addition, four vertical columns can be filled with seawater to submerge the platform during adverse weather conditions. The SMF has a self-supporting power supply consisting of four robust solar panels of 110Wh and a wind turbine to feed batteries.

As part of the ULTFARMS project technical performance of the system and the growth performance of mussels was monitored. The platform and longlines were monitored remotely daily using two onboard cameras. ROV (remotely operated vehicle) underwater images were obtained for automatic identification of species on the mussel lines to determine mussel presence, size and biodiversity. In addition, mussel samples were collected for analysis of contaminants.

The SMF was deployed in a highly exposed environment, which resulted in repeated longline failure. These issues proved challenging to address due to the continuously changing currents (direction and velocity) at the site, where no slack tide occurs. The main challenge encountered relates to the system's structural robustness. Following each component failure, the affected part was analysed, strengthened or redesigned. However, this often resulted in subsequent failures of other components. For instance, after the initial diabolo malfunctioned, a reinforced version was developed. When the longline subsequently broke, it was replaced with a Dyneema longline. Consequently, the system is still in an iterative phase of assessment and improvement. Upgrades were also required to the monitoring system. The existing power supply was enhanced with an additional battery to ensure sufficient energy storage. The onboard computer was replaced with a more robust model. These improvements have resulted in a stable and reliable monitoring and power supply system. Another challenge is procuring suitable support vessels. All vessels must be registered within the windfarm's operational system, and the windfarm operator and the Dutch Coastguard both require a five-day notice period.

As the longlines entangled before harvesting no culture cycle was completed. However, mussels from a test rope were collected 7 November 2024. At 6 months old, their average shell length was 44 mm and meat content 26%. The mussel samples for contaminant analysis were collected during two monitoring periods (7 November 2024 and 6 March 2025). No exceedances of the maximum levels for contaminants in were were observed. All concentrations remained well below the limits established in Regulation (EU) 2023/915 on maximum levels for certain contaminants in food.

Underwater videos were made of the droppers to determine mussel presence, size and biodiversity showed that the dropper ropes on the longlines did not catch a lot of mussel seed. The type of rope used for the droppers was different from the test ropes. The dropper ropes did not have the loops that the test rope had. This may have caused the difference in mussel yield. A test with the ROV on an inshore longline farm demonstrated automatic identification of species on the mussel lines.

Acknowledgment

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COMPARATIVE SPATIAL TRANSCRIPTOMICS OF *Aeromonas veronii* INFECTION IN EUROPEAN SEABASS SPLEEN

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Introduction

Aeromonas veronii is an important emerging bacterial pathogen of European seabass (*Dicentrarchus labrax*) aquaculture in the Mediterranean and is associated with substantial morbidity and mortality (Smyrli et al., 2017). A characteristic lesion of the disease is the formation of splenic abscesses, localized inflammatory foci containing host immune cells and viable bacteria. These structured lesions offer an excellent system for studying infection biology in situ. Within the Cure4Aqua project we used a seabass *A. veronii* challenge model to combine gross pathology, histology, scanning electron microscopy (SEM) and high-resolution spatial transcriptomics. The aim was to compare infected and uninfected spleen tissue and to define the spatial organization of the host response inside and around infection-associated lesions.

Materials and Methods

Forty-five healthy European seabass of approximately 130 g were distributed into three 350 L tanks. One group served as an uninfected control, while two groups were exposed by immersion for 2.5 h to 10^5 CFU mL⁻¹ of two *A. veronii* strains: a typical motile, pigment-producing strain (PDB) and a non-motile, non-pigmented strain (NS). Fish were monitored for clinical signs, and moribund fish were euthanized with an overdose of MS-222. Organs were collected for bacteriology, pathology, histology, SEM and spatial transcriptomics. Histology was performed at Pathovet AG, SEM at the University of Crete and HCMR, and spatial transcriptomic analyses at the Functional Genomics Center Zurich. Spatial transcriptomics was performed on spleen sections using the Visium HD 3' platform (10x Genomics). Infected spleen sections containing visible lesions were compared with an uninfected control spleen with normal histology.

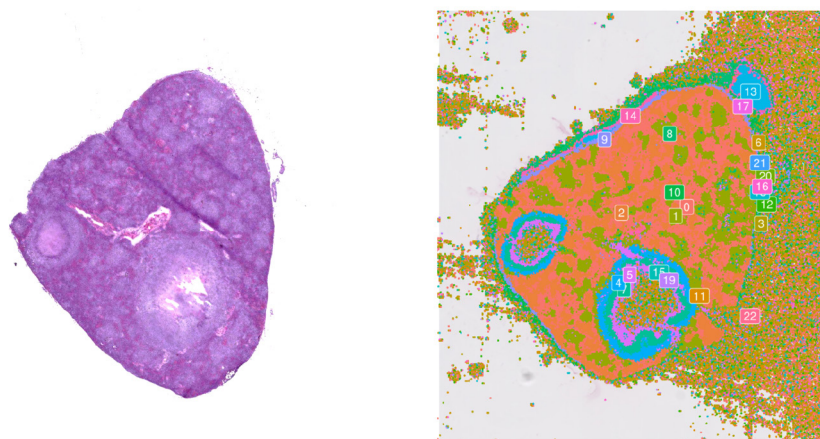


Figure 1. Left: spleen of a fish challenged with *Aeromonas veronii* NS with the characteristic lesions. Right: The spatial transcriptomic map of the same sample

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Results

Both *A. veronii* strains produced typical disease lesions within 7 days post-challenge, including epidermal haemorrhages, and abscesses in various internal organs such as spleen, liver kidney and brain. Bacteria were re-isolated from affected fish and confirmed by PCR. Histology showed severe splenic pathology in infected fish, while the uninfected control spleen showed normal tissue organization. SEM confirmed the presence of bacterial cells within splenic lesions and showed bacteria in close association with host phagocytic cells, supporting the interpretation that abscesses represent active host-pathogen interaction sites.

Spatial transcriptomics resolved infected spleen tissue into distinct molecular domains rather than a uniform organ-wide response. Lesion-associated regions showed strong enrichment of inflammatory, macrophage/myeloid, phagocytic, lysosomal and complement-related signatures. As examples, infected spatial domains included markers consistent with acute inflammation and cytokine signaling (*il1b*, *tnfa*, *cxcl8*), complement activation (*c3*), phagocyte activation and antimicrobial defense (*nos2*, *irg1*), tissue remodeling (*mmp9*), acute-phase response (*saa*) and cellular stress (*hsp70*). These markers supported active innate immune recognition, bacterial clearance mechanisms and local inflammatory activation. Surrounding tissue showed additional signatures associated with tissue stress, extracellular-matrix remodeling, stromal organization and repair. In contrast, the uninfected control spleen provided a spatial baseline of normal splenic architecture, without the strong focal inflammatory and remodeling domains observed in infected tissue.

Comparative analysis of infected and control spleens indicated that *A. veronii* infection reorganizes the splenic microenvironment into localized infection niches. These niches contained inflammatory and phagocytic cores surrounded by regions of metabolically active and remodeling tissue. The spatial data also showed that important disease-related signals were concentrated in specific tissue areas and would be partly diluted in whole-organ analyses. This spatial resolution is particularly useful for fish health studies because it connects molecular immune responses directly with lesion morphology and tissue damage.

Discussion

This study demonstrates that *A. veronii* infection in European seabass spleen is spatially organized. The combination of pathology, histology, SEM and spatial transcriptomics showed that splenic abscesses are not simple areas of tissue destruction, but structured infection microenvironments where bacteria, phagocytic cells, inflammatory pathways and tissue-remodeling responses interact. The comparison with an uninfected control spleen was important because it separated normal splenic organization from infection-associated molecular changes. For aquaculture, these findings are relevant because they provide tissue-architecture-level markers of disease progression and host response. Spatially resolved inflammatory, phagocytic, complement and remodeling signatures could be used in future challenge studies to evaluate disease severity, compare bacterial strains and assess whether interventions reduce lesion-associated pathology. By linking molecular signatures with histological and ultrastructural evidence, this approach offers a practical platform for understanding aeromonad disease mechanisms and for supporting targeted health-management strategies in Mediterranean seabass aquaculture.

Acknowledgment

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FUNCTIONAL NUTRITION TO ENHANCE CLIMATE STRESS RESILIENCE IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Climate change is increasingly exposing rainbow trout (*Oncorhynchus mykiss*) to heat stress and hypoxia, compromising performance, survival, and product quality. Nutritional strategies that enhance resilience are therefore critical. To address this challenge, we evaluated the efficacy of krill meal (KM) as a functional feed ingredient in three trials under climate-relevant stress conditions.

Materials and Methods

Three trials were conducted in Turkey, France, and India using juvenile trout fed diets with 2.5–10% KM for 10–12 weeks prior to stress exposure. Fish were challenged with (i) elevated temperature (14°C to 23°C), (ii) hypoxia (<6 mg L⁻¹), and (iii) acute thermal extremes (31°C and 0.2°C). Growth, feed utilisation, survival, digestive enzyme activity, pigmentation, and proteomic responses were assessed.

Results and Discussion

Across all trials, KM inclusion consistently improved feed intake, growth performance, and survival under stress conditions. Enhanced digestive enzyme activity and antioxidant capacity indicated improved metabolic responses. KM also helped maintain fillet pigmentation under hypoxia.

Proteomic analysis revealed upregulation of proteins associated with oxygen transport, antioxidant defence, and immune function, supporting improved physiological adaptation to environmental stress. These responses align with the nutrient profile of KM, which delivers phospholipid-bound omega-3 fatty acids, natural antioxidants, and key micronutrients such as choline that contribute to improved stress resilience.

Conclusion

Krill meal acts as a multi-functional nutritional strategy to enhance resilience in rainbow trout, supporting performance and robustness under climate-related stress conditions.

KRILL MEAL REDUCES THE IMPACT OF ENTEROCYTOZOOM HEPATOPENAEI (EHP) AND IMPROVES PERFORMANCE IN PACIFIC WHITE SHRIMP (*Penaeus vannamei*)

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Introduction

Hepatopancreatic microsporidiosis (HPM), caused by *Enterocytozoon hepatopenaei* (EHP), is a major constraint in shrimp aquaculture, leading to growth retardation and reduced performance. As no effective pharmaceutical treatment is currently available, nutritional strategies to support resilience are of interest. This study evaluated krill meal (KM) as a functional dietary ingredient to mitigate the impact of EHP infection in Pacific white shrimp (*Penaeus vannamei*).

Materials and Methods

A controlled tank trial was conducted using juvenile shrimp (4–5 g) fed diets containing 3%, 6%, and 9% KM for eight weeks, alongside infected and non-infected control groups. In an additional challenge setup, shrimp were pre-fed either a control diet or a 6% KM diet for 25 days prior to EHP exposure via ingestion of infected hepatopancreatic tissue. Growth performance, feed conversion ratio (FCR), survival, hepatopancreatic histology, pathogen load (qPCR), and proteomic responses were evaluated.

Results and Discussion

KM inclusion significantly improved growth, FCR, and survival compared to control groups ($p < 0.001$), with the strongest responses observed at 6% and 9% inclusion levels. Histological analysis showed a higher abundance of R cells in KM-fed shrimp, indicating improved hepatopancreatic functional capacity ($p < 0.001$).

Quantitative PCR analysis demonstrated a reduction in EHP copy number in KM-fed groups ($p < 0.001$), suggesting improved ability to cope with infection. Proteomic profiling indicated modulation of immune-related and stress-response proteins, consistent with enhanced physiological responses observed in performance outcomes.

Conclusion

Krill meal acts as a functional nutritional strategy to support shrimp performance and hepatopancreatic condition under EHP challenge. KM inclusion in diets contributes to improved growth, survival, and reduced pathogen load, supporting a nutritional approach to managing EHP in shrimp production systems.

SELECTIVE BREEDING ALTERS PHOSPHORUS REQUIREMENTS OF RAINBOW TROUT – A MODEL INTEGRATING BREEDING AND FISH NUTRITION

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Introduction

Advances in selective breeding and fish nutrition have markedly improved performance of farmed fish. Nutrient requirements of fishes are typically estimated using costly dose–response trials, and the requirements are often treated as static despite known context-dependent variation. Selective breeding has improved feed efficiency, body composition and nutrient retention, potentially altering nutritional needs, yet feed formulation rarely accounts for these genetic changes. Factorial models that link fish traits to nutritional requirements exist (Lupatsch et al. 1998), but an integrated multitrait framework is lacking. Dietary phosphorus (P) is essential for growth and skeletal development but contributes to eutrophication, and hence P levels in aquafeeds have been actively reduced in some countries. Balancing adequate dietary P with environmental sustainability remains a major challenge. This study (Kause et al. 2026) aimed to: 1) Develop traits-to-feed equations linking genetic trait changes to dietary P requirements of rainbow trout; 2) Apply it to the genetic improvement of traits in the Finnish rainbow trout breeding programme, and 3) Forecast future dietary P needs.

Material and Methods

Traits-to-feed equations link trait changes to feed formulation using mass balance principles. Body P depends on fillet yield (YLD), viscerosomatic index (VSI), and P content of fillet ($fillet_p$), viscera ($viscera_p$) and other parts of the body ($other_p$):

$$body_p = fillet_p \times YLD + viscera_p \times VSI + other_p \times (1 - YLD - VSI) \quad (1).$$

Optimal digestible dietary P can be calculated from feed conversion ratio (FCR), apparent digestibility coefficient of P (ADC_p = digestible P / total P in feed), efficiency of P retention (RE_p = g P gained / g P intake), and $body_p$ as defined in equation 1:

$$diet_{DP} = \frac{1}{FCR} \times \frac{ADC_p}{RE_p} \times body_p \quad (2).$$

Genetic trends of FCR, YLD, and VSI from 23 year-classes of the Finnish breeding programme (1992–2015; 547,246 fish) were used (Kause et al. 2026). To forecast future dietary P needed, genetic trends of the traits were projected to 2050, and the traits-to-feed equations were applied on the trends under three scenarios of P retention efficiency.

Results and Discussion

Changes in YLD and VSI had modest, linear effects on dietary P (Fig. 1a), whereas FCR had a strong, nonlinear effect – improving FCR substantially increased dietary P requirements (Fig. 1b). The average annual genetic improvement (in %) in the Finnish breeding programme was estimated to be -0.632% for FCR, +0.082% for fillet YLD%, -0.749% for VSI%, and 1.63% for body weight. Between birth years of 1992 and 2015, FCR has been genetically improved from 1.34 to 1.14, fillet YLD% from 64.7% to 65.3%, and VSI% from 11.1% to 10.7%. When equations 1 and 2 were applied over the genetic trends, the required dietary P were elevated across years (Fig. 2a), with future projections indicating a ~28% increase by 2050 if retention efficiency remains unchanged (Fig. 2b).

The model links genetic improvement directly to dietary requirements. FCR is the main driver of nutrient needs, while body composition has smaller effects. Fish with low FCR require more nutrient-dense feeds. Selective breeding has increased optimal dietary phosphorus requirements. Current phosphorus levels in feed may already be near or below optimal, and future increases may create tension between environmental goals and fish nutrition. Improving phosphorus retention efficiency will be critical. The traits-to-feed framework integrates genetics and nutrition to predict dietary needs.

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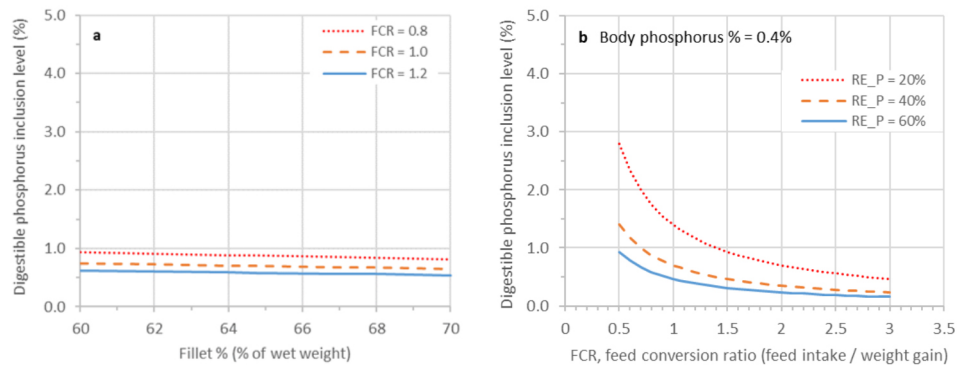


Figure 1. Effect of traits on optimal feed phosphorus levels, under different a) fillet yield (%) levels, and b) feed conversion ratios (FCR), when also phosphorus retention efficiency (RE_P) can differ.

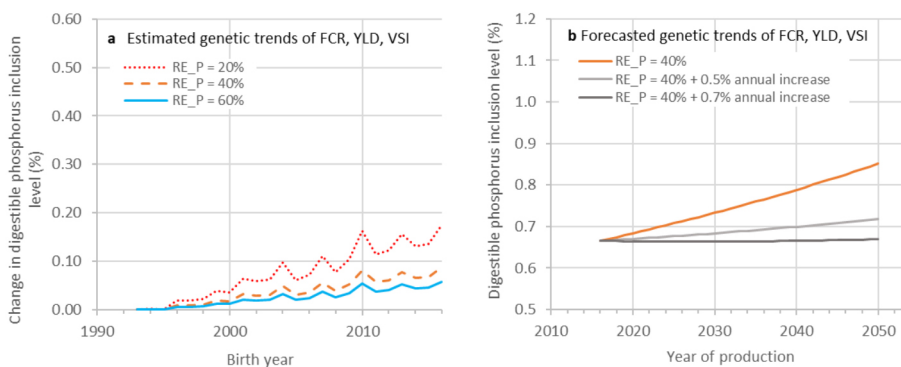


Figure 2. Effect of genetic trends in FCR, fillet yield (YLD) and viscerosomatic index (VSI) on optimal feed phosphorus levels, under different phosphorus retention efficiency levels (RE_P), a) in 1993-2016 based on estimated and b) in 2016-2050 based on forecasted changes in FCR, YLD and VSI due to breeding.

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EFFECT OF DIETARY MORINGA (*Moringa oleifera*) LEAF POWDER SUPPLEMENTATION ON INTESTINAL AND LIVER HISTOMORPHOLOGY IN STERLET (*Acipenser ruthenus*)

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Introduction

The use of functional feed additives in aquaculture offers new opportunities to improve fish health, welfare, and production performance (Onomu and Okuthe, 2024). Among plant-derived additives, moringa (*Moringa oleifera*) is of particular interest due to its high nutritional value and the presence of bioactive compounds with potential beneficial effects on metabolism, immune response, antioxidant status, and tissue condition (Saleh et al., 2025). Previous studies in fish have shown that dietary inclusion of *M. oleifera* leaf powder may improve growth performance, serum lipid profile, intestinal morphometry, and immune-related responses (El-Kassas et al., 2020). However, its influence on the digestive system and liver structure in sturgeon species remains insufficiently described. This study aimed to evaluate the effect of dietary supplementation with moringa leaf powder on the histological and histomorphometric characteristics of the anterior intestine, spiral intestine, and liver in sterlet (*Acipenser ruthenus*).

Materials and Methods

Sterlets were fed for 10 weeks with experimental diets containing different levels of moringa leaf powder supplementation: control diet without moringa addition (M0%), diet supplemented with 2.5% moringa (M2.5%), and diet supplemented with 5% moringa (M5%). Samples of the anterior intestine, spiral intestine, and liver were collected for histological analysis. Tissue sections were stained with haematoxylin and eosin and subjected to qualitative histopathological assessment and quantitative histomorphometric measurements. In the intestinal sections, fold height, epithelial height, supranuclear enterocyte height, and lamina propria width were measured. In the liver, hepatocyte area (HA), hepatocyte nuclear area (HNA), and hepatonuclear index (HNI) were determined.

Results

No differences in fish growth rates were observed among the studied groups. Qualitative analysis of the anterior intestine showed only mild infiltration of immune cells in the mucosa, while the spiral intestine displayed a typical structure for the species, with well-developed intestinal folds and no histopathological alterations. Liver samples were characterized by variable lipid accumulation (the lowest in fish from the M2.5% group), visible as lipid vacuoles in the cytoplasm of hepatocytes.

Histomorphometric analysis revealed that the highest intestinal folds and epithelial height in both the anterior and spiral intestine were observed in fish fed the diet supplemented with 5% moringa. In the anterior intestine, the greatest supranuclear enterocyte height and lamina propria width were recorded in the control group. In the spiral intestine, supranuclear enterocyte height was also highest in the control group, whereas lamina propria width was highest in the M5% group. Liver analysis showed that hepatocyte area was greatest in the control and M5% groups, while hepatocyte nuclear area reached the highest values in the M5% group. The hepatonuclear index was higher in both moringa-supplemented groups compared with the control.

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Discussion

The obtained results indicate that dietary supplementation with moringa leaf powder, particularly at the 5% level, may positively influence the histomorphological condition of the digestive tract in sterlet. Increased intestinal fold height and epithelial height in the M5% group suggest an enlarged absorptive surface and potentially enhanced digestive capacity, as gut histomorphological endpoints are commonly used to assess intestinal efficiency, nutrient absorption, and fish welfare status (De Marco et al., 2023). Similar effects of *M. oleifera* leaf powder on intestinal morphometric parameters, including increased villus length and width, have been reported in Nile tilapia (El-Kassas et al., 2020). Although lipid accumulation was observed in all examined livers, moringa supplementation was associated with favourable changes in liver histomorphometric parameters. This may correspond with previous observations indicating that moringa leaf powder can affect lipid metabolism and reduce serum lipid parameters in fish (El-Kassas et al., 2020). The increased hepatonuclear index in supplemented groups may reflect altered hepatocyte metabolic activity and improved liver condition. Overall, these findings suggest that 5% dietary moringa leaf powder supplementation may be a promising feeding strategy for supporting intestinal and hepatic health in sterlet.

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BIOFLOC TECHNOLOGY-BASED NUTRITIONAL STRATEGIES IN BROWN SHRIMP (*Farfantepenaeus aztecus*) CULTURE: A SYNTHESIS OF PROTEIN OPTIMIZATION, PREBIOTIC, AND SYNBIOTIC APPLICATIONS

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Introduction

Shrimp farming is one of the most valuable sectors of global aquaculture, but commercial production is still mainly based on a limited number of species. Therefore, alternative penaeid species are needed to support production diversity and reduce risks related to disease, environmental stress, and market dependency. Brown shrimp (*Farfantepenaeus aztecus*, also referred to as *Penaeus aztecus* in recent literature) is a promising candidate due to its commercial value, adaptation capacity, and recent records from the Mediterranean Sea. However, information on its culture performance and nutritional requirements remains limited. Biofloc technology (BFT) is a sustainable culture approach in which microbial aggregates contribute to water quality control and may also serve as an additional nutritional source for shrimp. In BFT systems, diet formulation and microbial dynamics are closely linked. Therefore, protein level, prebiotics, probiotics, microalgae-based ingredients, and synbiotic combinations should be evaluated together with growth, feed utilization, immune response, and gut microbiota.

This paper synthesizes three recent studies conducted with brown shrimp under BFT conditions (Genc et al., 2024a, 2024b; Kaya, 2025). These studies focused on dietary protein optimization, mannan oligosaccharide supplementation, and enriched synbiotic diets containing probiotics, *Arthrospira*, and MOS. Together, they provide a practical basis for developing BFT-based feeding strategies for brown shrimp.

Biofloc-Based Nutritional Strategies for Brown shrimp

Biofloc technology links water quality, microbial biomass, and nutrition, and therefore provides a useful culture model for brown shrimp. In the reviewed studies, juvenile brown shrimp adapted well to BFT conditions and responded positively to targeted dietary strategies.

In the protein optimization study, 29%, 32%, 35%, and 38% dietary protein levels were tested on brown shrimp. Growth performance improved up to 35%, suggesting that this level can be used as a practical reference for further feeding studies under BFT conditions (Genc et al., 2024a).

In the MOS study, 0 - 4 g kg⁻¹ mannan oligosaccharide was evaluated for 90 days. The best overall response was observed at 3 g kg⁻¹ MOS, with improvements in growth, feed utilization, digestive activity, immune response, and gut microbiota-related parameters of brown shrimp (Genc et al., 2024b).

In the enriched synbiotic study, probiotics, *Arthrospira*, and MOS were tested individually and in combination. The combined enriched synbiotic diets produced stronger growth and immune-related responses than single or dual additive applications, indicating that functional feed strategies can strengthen BFT-based brown shrimp culture (Kaya, 2025).

Conclusion

The reviewed studies collectively indicate that biofloc technology can provide a practical and sustainable culture framework for brown shrimp. Beyond water quality management, BFT systems link nutrition, microbial dynamics, digestion, and immune status. From this perspective, the culture success of brown shrimp depends not only on the system itself, but also on well-planned dietary strategies. Optimized protein nutrition and functional additives such as MOS, probiotics, and *Arthrospira* may contribute to more efficient and healthier production. These completed studies also provide a scientific background for our ongoing TÜBİTAK 1004 Tr.Aqua project, Project No. 22AG009. Within APYOK - Project 2, we are currently evaluating PHB-enriched biofloc technology and antimicrobial peptide (AMP)-based functional feed strategies in Pacific white shrimp (*Litopenaeus vannamei*) and brown shrimp (*Farfantepenaeus aztecus*) culture.

(Continued on next page)

Acknowledgment

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DOES ATLANTIC SALMON NEED SUNLIGHT? NEW FINDINGS ON VITAMIN D METABOLISM

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Introduction

Vitamin D₃ is crucial for mineral homeostasis, skeletal health, and immune function in vertebrates. Historically, fish were thought to rely solely on dietary sources for vitamin D rather than synthesizing it via UV exposure. However, there is limited information about typical plasma vitamin D levels in farmed salmon under various production and seasonal conditions. The transition from marine to plant-based raw materials raises further concerns about vitamin D intake in farmed fish. To address these gaps and document dietary supplementation in commercial settings, several studies have been conducted and will be presented.

Methods

- Trial 1: Blood sampled from two commercially farmed fish groups in open pens in seawater from June 2023 to Dec 2024 in Rogaland, Southern Norway*
- Trial 2: Whole-body analyses of a fish group of juvenile salmon kept in freshwater indoors and outdoors for 52 days*
- Trial 3: Muscle (NQC) sampled from fish fed a standard diet with 4-5000 IU/kg vitamin D₃ or an experimental diet with 35-45000 IU/kg vitamin D₃. Fish (1-4 kg) were grown at a commercial seawater farm in Rogaland, Southern Norway**

Results and Conclusion

The first trial combined observational data from commercial farming sites with a controlled dietary intervention to characterize the concentrations and variability of cholecalciferol (vitamin D₃), 25hydroxycholecalciferol [25(OH)D₃], and 1,25dihydroxycholecalciferol [1,25(OH)₂D₃] in plasma of Atlantic salmon (*Salmo salar*). Substantial variation in vitamin D metabolite levels was observed both within populations and between fish groups. Monitoring an outdoor farm over 18 months revealed a distinct seasonal pattern between vitamin D levels and day length, showing significantly higher levels during the summer months compared to winter months.

In a follow-up trial (Trial 2), indoor-raised fish were divided into two groups, one remained indoors, while the other was transferred outdoors for 52 days. Both groups received the same commercial diet, yet the outdoor group exhibited a fivefold increase in whole-body vitamin D content.

In a third trial, metabolic responses to increased dietary vitamin D were assessed and salmon were fed either a standard diet (~4000 IU/kg vitamin D₃) or a diet with a tenfold higher inclusion (~40 000 IU/kg vitamin D₃) for 32 weeks under commercial conditions. Fish tolerated the high dietary vitamin D without adverse effects on growth or mortality. Elevated dietary inclusion increased concentrations of vitamin D₃ in plasma, liver, and muscle, while hydroxylated metabolites showed more moderate responses and seasonal variation.

In conclusion, our findings demonstrate that vitamin D status is strongly influenced by season and provide evidence for endogenous vitamin D synthesis in Atlantic salmon induced by natural sunlight. Furthermore, winter dietary fortification can achieve tissue vitamin D concentrations comparable to those observed during summer. Nutritional quality of salmon fillet can be improved as elevated dietary vitamin D enhanced tissue deposition. Dietary fortification of vitamin D is particularly important for fish raised indoors and during seasons with limited sunlight exposure outdoors.

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ROLE OF CATALASE IN MITIGATING OXIDATIVE STRESS IN *Micropterus salmoides* (Largemouth Bass) FISH: AN IN-SILICO ANALYSIS

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Catalase (CAT) is a critical antioxidant enzyme in largemouth bass (*Micropterus salmoides*) that converts toxic hydrogen peroxide (H_2O_2) into water and oxygen. It acts as a primary cellular defender, mitigating oxidative stress and preventing tissue damage during environmental challenges such as thermal shifts. Catalase is primarily localized in peroxisomes and mitochondria. It catalyzes the rapid decomposition of reactive oxygen species (ROS) into water and oxygen, maintaining cellular redox homeostasis. By clearing H_2O_2 efficiently, the enzyme protects lipid membranes, proteins, and DNA from oxidative degradation, thus maintaining cellular integrity. Largemouth bass frequently encounter aquaculture and environmental stressors like crowding, handling, transportation, and changing water temperatures. Catalase assists in managing these conditions.

The Catalase (NCBI accession number: XP_038560904.1) from *Micropterus salmoides*, comprising 527 amino acids, was retrieved from the UNIPROTKB database. In silico analysis of its physicochemical properties using ProtParam revealed an isoelectric point of 7.65, a molecular weight of 59919.52 Da, an instability index of 29.72, an aliphatic index of 62.90, and a GRAVY value of -0.583. Alanine is present in the highest number 38 (7.2%) and total number of negatively charged residues (Asp + Glu) were 59 in numbers. On the other hand, the total number of positively charged residues (Arg + Lys) was present to be 60 in numbers. Conserved domain (CD) analysis identified the catalase_clade_3 (cd08156) domain spanning amino acid residues 68 to 497, confirming its functional identity. Three-dimensional structural modeling of the protein was performed using AlphaFold2 available at <https://neurosnap.ai/>. Among the five predicted models, model 1 exhibited a mean local distance difference test (LDDT) score of 94.47, indicating very high structural accuracy. Further, this model was validated for its quality by Ramachandran plot which showed 97.6% residues in the most favored and additional allowed regions, ERRAT illustrated 88.7 score, which revealed a good structure quality of the model.

Furthermore, the NJ phylogenetic tree was generated using MEGAX analysis, which revealed that out of 21 homologous proteins, catalase in *Siniperca chuatsi* was observed as the closest evolutionary relative, supported by a weak bootstrap value.

PATHOGEN PROLIFERATION RISK IN AQUATIC SYSTEMS: DATA-DRIVEN CAUSAL INFERENCE OF ENVIRONMENTAL DRIVERS TOWARD ECOLOGICAL MANAGEMENT

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Introduction

Advancements in smart aquaculture have intensified the demand for data-driven approaches to identify disease risks, yet conventional water-quality practices relying on real-time sensors and correlation analyses (Deng, 2019; Su et al., 2025; Tran et al., 2010) cannot resolve causal directionality or distinguish direct from indirect effects in tightly coupled aquatic ecosystems. To overcome these limitations, this study employs a three-tier analytical framework that progressively transitions from association to causation: first, Spearman rank correlation maps pairwise co-variations; second, SHapley Additive exPlanations (SHAP) values from Random Forest models quantify non-linear predictor contributions; and third, the Direct Linear Non-Gaussian Acyclic Model (DirectLiNGAM), integrated with bootstrapping and biological priors, explicitly infers the mechanistic causal pathways linking environmental stressors to pathogen dynamics.

Utilizing this framework, the study achieves two primary objectives: characterizing the temporal and seasonal dynamics of fish pathogens in olive flounder farms on Jeju Island, South Korea, relative to water temperature (WT) variations, and identifying the environmental factors causally governing opportunistic pathogen proliferation. Building upon a previous correlation-based predictive study (Choi et al., 2026), this work extends the analytical paradigm from prediction to causal inference, establishing a mechanistic blueprint for ecological pathogen control and providing a causal-discovery framework to support AI-assisted decision-making in intensive aquaculture systems.

Materials and Methods

Pathogen populations were monitored across eight distinct sampling campaigns per system, while environmental water-quality parameters were documented at a higher frequency. To establish a rigorous baseline independent of interpolation, Spearman rank correlations were computed using only these eight empirical data points. Conversely, for trend-attribution and causal-discovery frameworks (specifically SHAP and DirectLiNGAM) which necessitate a uniform temporal resolution. This technique aligns with established practices for reconstructing sparse ecological time-series data prior to modeling and calibration (Antonakos and Lambrakis, 2021; Bu et al., 2026; Cui et al., 2019).

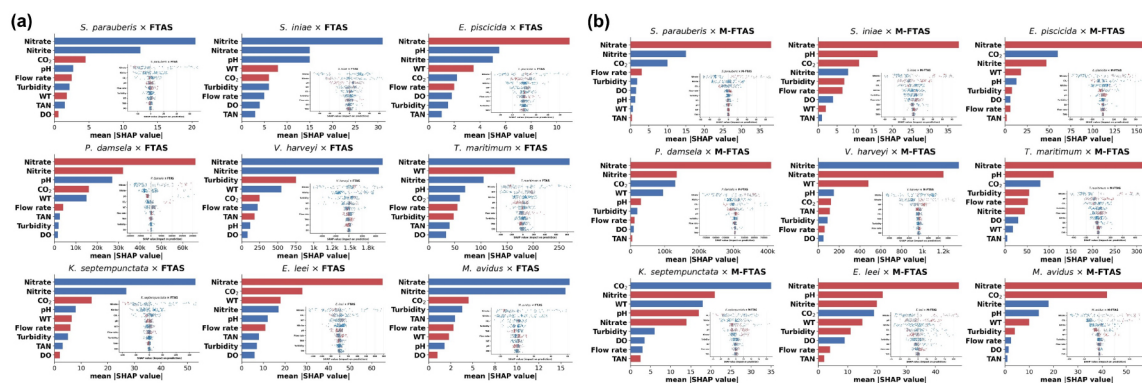


Fig. 1. Water-quality drivers of pathogen abundance determined by SHAP analysis in (a) FTAS and (b) M-FTAS. Mean absolute SHAP values are displayed as bars, with red and blue identifying positive and negative effects, respectively. The accompanying insets provide the distribution patterns of individual SHAP values.

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Results

Environmental drivers primarily redistributed competitive advantages among pathogens rather than uniformly suppressing them. The acidification axis (high CO₂/low pH) dominated direct influences, significantly suppressing *K. septe mpunctata* ($\beta = -1.02$), *E. leei* (-0.87), *S. iniae* (-0.36), and *S. parauberis* (-0.32), but promoting *M. avidus* (+0.39). Conversely, nitrogen species acted as major promoters: nitrate boosted *E. leei* (+1.25), *K. septe mpunctata* (+1.15), and *M. avidus* (+0.70), while nitrite enhanced *E. piscicida* (+0.43) and *P. damsela* (+0.40). Turbidity had weak negative effects.

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DEVELOPMENT OF A MICROORGANISM–MICROALGAE HYBRID CULTURE SYSTEM FOR ENHANCED MICROALGAL BIOMASS PRODUCTION

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Microalgae fix carbon dioxide and release oxygen through photosynthesis, yielding valuable metabolites such as glucose, glycerol, amino acids, and free fatty acids. In particular, their high lipid and protein content, rich in essential fatty acids and containing essential amino acids, respectively, make microalgae a key resource for functional food supplements and animal feed in the pharmaceutical and food industries. Despite these advantages, economically viable strategies for optimizing raw material production remain underexplored. Industrial-scale applications require further research to increase yield per unit area, reduce culture medium costs, standardize raw materials, and define optimal culture conditions.

In this study, we developed a microorganism–microalgae hybrid culture system designed to enhance microalgal raw material production, and used it to examine how carbon dioxide generated by microbial metabolism affects microalgal growth. The hybrid system yielded a higher cell density ($5.5 \times 10^4 \pm 2.3 \times 10^3$ cells/mL) than the microalgae-only bioreactor control ($4.0 \times 10^4 \pm 4.1 \times 10^3$ cells/mL). Carbon dioxide bio-fixation was also higher in the hybrid system (0.04 ± 0.01 g/L/d) than in the control (0.03 ± 0.01 g/L/d).

These results demonstrate the potential of the microorganism–microalgae hybrid culture system as a practical approach for enhancing microalgal raw material production toward industrialization.

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ANNUAL REPRODUCTIVE CYCLE OF FEMALE CHINESE SLEEPER *Perccottus glenii* (DYBOWSKI, 1877) (TELEOSTEI: GOBIIFORMES: ODONTOBUTIDAE), AN INVASIVE FISH SPECIES IN CENTRAL EUROPE

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The Chinese sleeper (*Perccottus glenii*) is an invasive fish species native to South Asia, occurring in Poland for over 20 years. It is a highly expansive species due to its ecological plasticity, reproductive potential associated with multiple spawning, high fecundity, and male care for the eggs.

Its presence negatively impacts the functioning of the ecosystem and threatens local fish species. The aim of this study was to characterize the annual reproductive cycle of female Chinese sleeper in a newly colonized habitat.

The study material consisted of 282 females collected from the Wilanówka River (a tributary of the Vistula River). The fish were collected once a month (every two weeks during the spawning season) using electrofishing. The age of the studied fish, assessed based on scale analysis, ranged from 0+ to 6+. The average length (SL) of the fish was 67 mm, or 6.38–1.85 mm. At age 1+, 90% of females matured, as did all older ones. The smallest female that laid eggs was 38 mm (age 1+).

Histological analyses of gonadal preparations performed using standard paraffin techniques indicated exceptionally long batch spawning in females, lasting from early April to mid-September. Peak spawning occurs between April and June. From October to February, the gonads of Chinese sleeper were in early vitellogenesis. Oocyte degeneration was visible in the gonads in March, September, and October. The highest GSI in females was recorded in May (average 8.7) and the maximum individual value was 20.3 (May). GSI in females during the spawning months reached high (10-18%) and low (2-6%) values due to batch spawning, depending on whether the female was pre-spawning (first batch, high GSI) or post-spawning.

Five classes of oocytes were distinguished in the female gonads, the largest of which had a diameter of 1.5 mm. The absolute fecundity of Chinese sleeper was 2971 eggs/female, and relative fecundity was 284 eggs/1 g of female weight. The number of oocytes increased with fish length and age.

Detailed histological analyses revealed that Chinese sleeper populations inhabiting newly colonized environments exhibit earlier sexual maturity (as early as age 1+) and a prolonged reproductive season compared with populations from the native range and with native fish species in the study area. Moreover, high female fecundity and batch spawning, which enhance offspring survival through nest maintenance and protection, further increase the species' reproductive potential. Together with pronounced environmental plasticity, these traits confer a strong competitive advantage and underpin the species' colonization success.

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SEASONAL, GEOGRAPHIC AND SIZE-DEPENDENT DRIVERS OF FILLET VITAMIN D₃ IN NORWEGIAN ATLANTIC SALMON (*Salmo salar*)

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Introduction

Vitamin D is crucial for fish health (Lock et al., 2009) and the nutritional value of salmon fillets. While dietary supply has long been considered the main source for teleosts (Lock et al., 2009; Soto-Dávila et al., 2020; recent experimental and field evidence indicates cutaneous UV-B driven synthesis contributes substantially in Atlantic salmon (Fossen et al., 2025; Husebø et al., 2025). This study examined whether season, latitude and fish size explain variation in fillet vitamin D₃ concentration across Norwegian commercial production of Atlantic salmon from 2005–2025.

Material and methods

The study comprises 1,473 samples of farmed Atlantic salmon (*Salmo salar*) collected year-round along the Norwegian coast between 2005 and 2025 by the Norwegian Food Safety Authority and the Institute of Marine Research (IMR). From each farm, five to ten market-size fish were taken, transported frozen (−20 °C) to IMR and processed according to standardized protocols. From 2011 onwards, muscle was sampled using the Norwegian Quality Cut (NQC; NS 9401), while samples from 2005, 2006 and 2010 used a whole-side fillet protocol; samples through 2022 were generally individual fish, with some pooling by area and from 2023–2025 three NQC cuts were pooled prior to analysis. Individual samples were homogenized, freeze-dried, re-homogenized to a fine powder and used for most analyses, whereas wet homogenates (prior to freeze-drying) were reserved for lipid and vitamin determinations. Wet material was stored at −80 °C until analysis, and freeze-dried powders were kept dry at room temperature pending further assays (Reksten et al., 2022).

Results

Three consistent patterns emerged from the data. First, a pronounced seasonality: fillet D₃ concentrations show an annual cycle, rising from spring and peaking in late summer/early autumn and falling to their lowest levels during mid-winter ($p < 0.01$). Second, a negative weight effect: D₃ declines with increasing fish weight, smaller fish contained higher $\mu\text{g}/100\text{ g}$ D₃ than larger fish ($p < 0.01$). Third, a modest but statistically significant positive latitude association: fillet D₃ increases slightly with higher latitude, maybe an effect that likely reflects a combination of slower growth and longer residence times in northern, colder waters and maybe site-specific optical conditions that favour deeper UV-B penetration ($p < 0.01$).

Smaller fish have a larger skin surface area relative to mass and therefore greater potential for UV-B driven synthesis. Because vitamin D metabolites in salmon turn over rapidly, these patterns imply practical management options: targeted winter supplementation, size-class specific feeding regimes with higher vitamin D input for larger fish (and dosing in the weeks before harvest), and production-system decisions (open surface cages receive substantial seasonal photogenic input, while submerged, covered or land-based systems will require greater or continuous dietary supply).

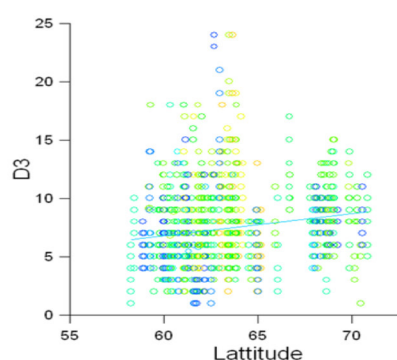


Figure 1: Vitamine D3 content increases for each latitude one moves north ($p < 0.01$).

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SEASONAL, GEOGRAPHIC AND SIZE-DEPENDENT DRIVERS OF FILLET VITAMIN D₃ IN NORWEGIAN ATLANTIC SALMON (*Salmo salar*)

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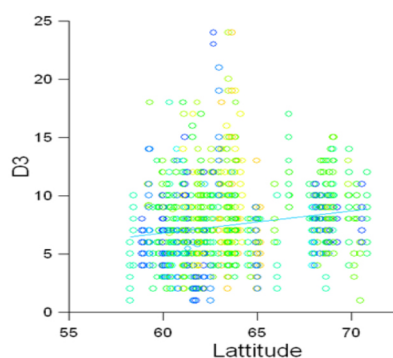


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DRIVERS OF ELECTRICAL ENERGY CONSUMPTION IN RECIRCULATING AQUACULTURE SYSTEMS

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Recirculating Aquaculture Systems (RAS) are efficient farming systems, but energy intensive. Few studies distinguish energy consumption between individual components or identify the factors driving demand. This study aims to characterise baseline energy demand and variability across RAS components. It furthermore quantifies the operational and environmental drivers of electrical energy use by analysing relationships between component-level energy use and production parameters.

Eight identical experimental RAS were run at the Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB) in Berlin, Germany, from May to August 2025 with African catfish (*Clarias gariepinus*). Smart energy sensors monitored all energy-consuming components during the 13-week feeding trial. Electrical energy use was recorded at system and component levels, as well as biological and environmental conditions. Component-level data were analysed to quantify baseline energy demand and variability among compartments. Regression and correlation analyses identified operational and environmental drivers of energy use. Energy use efficiency was calculated based on the biomass produced and energy used.

The results show that drumfilter energy use increased over time with growing biomass and that energy use for temperature control was highly variable. In total, temperature control dominated energy use at more than 60% of total consumption, followed by the blower (22.27%), trickling filter pump (10.58%), circulation pump (4.68%), and drumfilter (0.87%) (Figure 1). Correlation results indicate that electrical energy use efficiency in RAS strongly depends on external environmental conditions and fish growth performance.

By differentiating energy use among system compartments and identifying the main operational and environmental drivers, this study provides a basis for targeted optimisation of RAS design and operation, supporting more effective energy management strategies in aquatic animal farming.

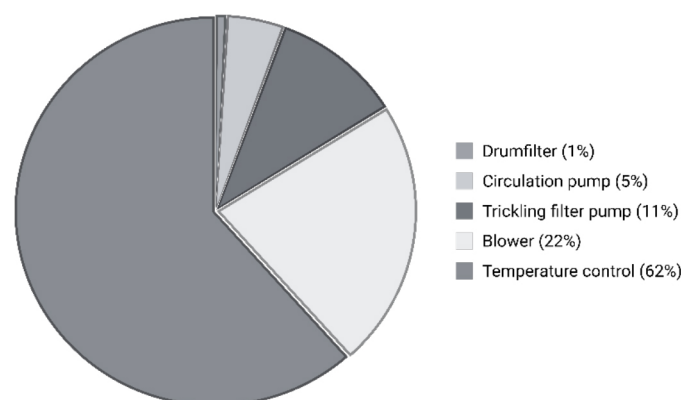


Figure 1. Electrical energy use across individual compartments in RAS, characterising the variability between the drumfilter, circulation pump, trickling filter pump, blower, and temperature control.

INNOVATIVE SUSTAINABLE AQUACULTURE PRACTICES: USING AQUACULTURE WASTE STREAMS AS FERTILIZER FOR MICROALGAE PRODUCTION – OPPORTUNITIES AND CHALLENGES

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The European Green Deal has recognised aquaculture and algae as promising solutions to achieving the goals of the Farm-to-Fork Strategy. However, despite its potential, the growth of aquaculture in Europe has been limited compared to the significant expansion observed in Asia. In this context, the EU-funded INNOAQUA project aims to pave the path towards the upcoming sustainable and diversified EU land-based aquaculture industry by demonstrating and mainstreaming innovative algae-based foods and solutions, using ecology, circularity, and digitalization approaches.

The project will demonstrate the benefits of algae, from the production in combination with fish aquaculture in a circular approach, to the formulation and production of innovative seafood products. INNOAQUA partners are developing integrated aquaculture systems combining fish and algae production. Research on microalgae-salmon RAS-IMTA systems (Recirculating Aquaculture Systems – Integrated Multitrophic Aquaculture) on our Norwegian demo site demonstrates that nutrients from fish farming can be reused, while valuable microalgae biomass can be produced simultaneously. New data on the microbiome of the system will be presented, as well as other water quality data. Another recent development within our approach to transform aquaculture side streams into high-value resources is the nutrient recovery from aquaculture sludge into fertilizing products for algae. Trials at lab-scale show that sludge can be processed to liberate more nutrients, especially phosphorus, which can subsequently be used by the microalgae as a nutrient source.

Though the technological results are all very promising, there are still barriers before full exploitation can be achieved, mainly in the form of regulatory challenges. Clarifying regulatory pathways and aligning classifications with their actual environmental and public and animal health impact could enhance the sustainable management of aquaculture effluents while supporting circular economy principles. Addressing these regulatory ambiguities is required to facilitate their responsible use, unlocking their potential as a resource rather than a disposal challenge.

SUSTAINED-RELEASE HORMONE DELIVERY MODULATES REPRODUCTIVE RESPONSES IN PIKEPERCH (*Sander lucioperca*)

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Introduction

Reliable control of reproduction is essential for efficient broodstock management and reliable gamete production in aquaculture (Zohar and Mylonas, 2001; Mylonas et al., 2010). In pikeperch (*Sander lucioperca*), reproductive dysfunction under captive conditions frequently compromises ovulation, spermiation and synchronization of gamete release, resulting in reduced reproductive performance (Migaud et al., 2013).

Hormonal induction using gonadotropin-releasing hormone analogues (GnRHa) is commonly employed to stimulate final oocyte maturation, ovulation and spermiation (Mylonas et al., 2010). However, conventional hormonal treatments are often limited by rapid peptide degradation and insufficient duration of endocrine stimulation (Carnevali et al., 2006).

Poly(lactic-co-glycolic acid) (PLGA) is a biodegradable and biocompatible polymer widely used in sustained-release formulations (Makadia and Siegel, 2011). Encapsulation of GnRHa into biodegradable microparticles enables prolonged hormone release and sustained activation of reproductive endocrine pathways during critical phases of gamete maturation (Matejkova and Podhorec, 2019; Knowles et al., 2022).

The present contribution evaluates sustained-release hormone delivery systems in pikeperch, with emphasis on ovulation induction, spermiation and endocrine responses under captive broodstock conditions.

Results and Discussion

This contribution investigates biodegradable microparticles as a sustained-release hormone delivery system modulating reproductive responses in pikeperch under captive broodstock conditions.

In female pikeperch, microparticles containing low doses of alarelin significantly improved ovulation success and spawning performance compared with conventional hormonal treatment. Sustained-release formulations prolonged endocrine stimulation during final oocyte maturation, modulated plasma sex steroid profiles associated with ovulation induction, and improved synchronization of ovulation while reducing the total hormone dose required for successful spawning.

In male pikeperch, sustained-release delivery of alarelin prolonged plasma testosterone responses compared with conventional hormonal induction protocols. Sustained endocrine activation was associated with maintenance of sperm motility parameters assessed by computer-assisted sperm analysis and improved temporal stability of sperm quality during the spawning period, indicating the potential to prolong the functional fertilisation window under hatchery conditions.

The PLGA delivery system was designed to protect peptide hormones from rapid enzymatic degradation and maintain sustained activation of reproductive endocrine pathways during critical phases of gamete maturation. This approach addresses one of the principal limitations of conventional hormonal induction protocols, namely the short duration of endocrine stimulation and insufficient synchronization of reproductive responses.

From a broodstock management perspective, sustained-release hormone delivery may improve temporal synchronization of ovulation and spermiation, reduce repeated broodstock handling and enhance reproductive reliability under intensive aquaculture conditions.

Conclusion

Biodegradable microparticles represent an effective platform for sustained endocrine stimulation in fish reproduction. Sustained-release formulations in pikeperch improve ovulation success, spawning synchronization and spermiation while modulating reproductive endocrine responses and maintaining sperm quality under captive conditions.

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Prolonged hormone release supported more stable activation of reproductive pathways during critical phases of gamete maturation and reduced limitations associated with conventional hormonal induction based on short-term endocrine stimulation.

The integration of pharmaceutical sustained-release systems into broodstock management may reduce repeated broodstock handling, improve the reliability of controlled reproduction and support more predictable juvenile production in intensive aquaculture.

Acknowledgment

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BIODEGRADABLE MICROPARTICLES FOR REPRODUCTIVE MANAGEMENT IN EMERGING AQUACULTURE SPECIES

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Introduction

Reliable control of reproduction is essential for efficient broodstock management and reliable juvenile production in intensive aquaculture (Zohar and Mylonas, 2001; Mylonas et al., 2010). In many emerging aquaculture species, reproductive dysfunction under captive conditions remains a major bottleneck limiting species diversification and commercial production (Migaud et al., 2013). Impaired ovulation, reduced spermiation and poor synchronization of gamete availability frequently compromise hatchery performance and juvenile output.

Hormonal induction using gonadotropin-releasing hormone analogues (GnRHa) is commonly employed to stimulate final oocyte maturation, ovulation and spermiation (Mylonas et al., 2010). However, conventional hormonal treatments are often limited by rapid peptide degradation and short endocrine stimulation, requiring repeated broodstock handling and increasing stress-related risks (Carnevali et al., 2006).

Poly(lactic-co-glycolic acid) (PLGA) is a biodegradable and biocompatible polymer widely used in pharmaceutical sustained-release formulations (Makadia and Siegel, 2011). Encapsulation of GnRHa into biodegradable microparticles enables controlled hormone release over several days and may prolong endocrine stimulation during critical phases of gamete maturation (Matejkova and Podhorec, 2019; Knowles et al., 2022).

The present contribution evaluates the application of biodegradable microparticles for reproductive management in several fish species, with particular emphasis on pikeperch (*Sander lucioperca*), a high-value species of increasing importance in European intensive aquaculture.

Results and Discussion

This contribution investigates biodegradable microparticles as a controlled hormone delivery platform for reproductive management in emerging aquaculture species, with particular emphasis on pikeperch (*Sander lucioperca*), for which comprehensive data are available for both sexes.

In female pikeperch, microparticles containing low doses of alarelin significantly improved ovulation success and reproductive performance compared with conventional hormonal treatment. Sustained hormone-release enhanced spawning induction while reducing the total hormone dose required.

In male pikeperch, microencapsulation of alarelin prolonged endocrine stimulation and maintained sperm motility characteristics under hatchery conditions, indicating the potential to improve synchronization and extend the functional fertilisation window.

The delivery system was designed to protect peptide hormones from rapid degradation and maintain effective hormone availability during critical phases of gamete maturation. This approach addresses one of the principal limitations of conventional gonadotropin-releasing hormone analogue treatments, namely the short duration of hormonal stimulation and the need for repeated broodstock handling.

In addition to pikeperch, this technology was successfully evaluated in peled (*Coregonus peled*), northern pike (*Esox lucius*), sterlet (*Acipenser ruthenus*) and bala shark (*Balantiocheilos melanopterus*). Positive reproductive responses across teleosts and sturgeons indicate broad applicability in species with diverse reproductive strategies and production requirements.

(Continued on next page)

From a practical perspective, controlled hormone delivery may improve synchronization of ovulation and spermiation, reduce handling stress and increase the reliability of juvenile production. By improving reproductive control under captive conditions, biodegradable microparticles may support species diversification and facilitate the introduction of new fish species into intensive aquaculture systems.

Conclusion

Biodegradable microparticles represent a versatile platform for the controlled delivery of reproductive hormones in fish. Results obtained in pikeperch (*Sander lucioperca*) and additional fish species demonstrate that sustained-release formulations can improve reproductive synchronization, reduce broodstock handling and enhance the reliability of juvenile production under captive conditions. The integration of pharmaceutical sustained-release systems into broodstock management offers a promising approach for overcoming reproductive bottlenecks in emerging aquaculture species. By improving reproductive control and hatchery reliability, biodegradable microparticles may support species diversification and facilitate the introduction of new fish species into intensive aquaculture systems.

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NEXT GENERATION AQUACULTURE PLATFORM SYSTEM: AQUA COLONY

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Introduction

The Japanese aquaculture industry faces a dual crisis: a volatile surge in the cost of fishmeal, primarily imported, and a chronic labor shortage exacerbated by an aging workforce. Traditional maritime farming relies on experience-based feeding and high-emission manned vessel logistics, which are increasingly unsustainable. To secure future food systems, a transition from fragmented, manual operations to an integrated autonomous platform is strategically vital. This research presents the “Aqua Colony” concept, a self-sustaining ecosystem inspired by space colony architectures. Unlike current smart aquaculture efforts that focus on isolated monitoring tasks, this platform achieves end-to-end automation—from the biological production of insect-based feed to robotic delivery and harvest management. By integrating decentralized land-based production with sea-based autonomous systems, Aqua Colony creates a resilient, closed-loop model for the cultivation of yellowtail.

Methodology: The Aqua Colony Ecosystem

The platform architecture utilizes a hierarchical control structure where a central Planner acts as the master controller, directing the sub-AIs of three physical components: the Worm Pod, Smart Drone, and Smart Cage.

Decentralized Feed Production (Worm Pod): To mitigate the risks of centralized large scale mealworm factories, we developed a “Virtual Large-Scale Plant” consisting of decentralized 9 m² units. This architecture is optimized for Japan’s narrow topography, utilizing local resources such as food waste from restaurants and dried vegetable scraps to rear mealworms. The “Worm Pod 4 Layers Architecture” consists of an IoT Layer for environmental control, a Sensing Layer for data acquisition, a Recognition Layer utilizing YOLOv8 to identify larval growth stages and notify of “harvest windows,” and a Virtualization Layer for remote fleet management (Kobayashi et al., 2026).

Autonomous Delivery and Feeding (Smart Drone): Transport between land and sea is managed by a multi-drone system using a “Demand-Driven, Energy-Aware Distributed Coordination” mechanism. Navigation employs GPS for transit and ArUco markers for precision station-keeping over cages, correcting GPS-induced drift. To ensure collision-free operations, we implement the Artificial Potential Field (APF) method, allowing drones to coordinate distribution based on real-time hunger indices and battery states (Yanagisawa et al., 2026).

Real-time Assessment of Fish School Activity and Fishing Net Maintenance (Smart Cage): The Smart Cage serves as the sensory hub (Fukae et al., 2024). Live underwater video is captured via OBS Studio and processed through a GPU-accelerated pipeline. By shifting Optical Flow calculations from the CPU to the GPU and utilizing parallel processing across four concurrent programs, we eliminated computational bottlenecks. A fixed synchronization delay of 3.0 s was implemented to align fish behavior vectors with Rose Diagram visualizations. A Support Vector Machine (SVM) classifies these behaviors into “Hungry” or “Satiated” states. Additionally, an autonomous underwater drone performs net inspections, using contour intensity analysis to generate 5-level heatmaps of algae accumulation for proactive maintenance.

Results and Discussion

Component trials have validated the feasibility of the Aqua Colony framework. The Worm Pod demonstrated commercial viability with an implementation cost of approximately 1.27 million JPY, successfully converting vegetable waste into high-protein biomass. The Smart Drone achieved stable “Takeoff-Flight-Feed-Return” cycles using the MQTT protocol, though a 50% precision rate in heavy wind indicates a need for enhanced control algorithms. The Planner’s predictive analytics for yellowtail market prices proved highly accurate, enabling strategic harvesting (Miyajima et al., 2026). Future research will focus on transitioning from automated to fully predictive autonomy. We are currently considering a Multi-Agent Partially Observable Markov Decision Process (MA-POMDP) and World Models to handle the inherent partial observability (e.g., water turbidity, weather interference) of marine environments. This will enable the system to perform proactive, collaborative decision-making without human intervention, ensuring food security in an era of environmental uncertainty.

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Acknowledgment

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DIETARY NUCLEOTIDES ON PERFORMANCE, NITROGEN STRESS RESISTANCE AND RETURN ON INVESTMENT FOR PACIFIC WHITE SHRIMP, *Penaeus vannamei*, RAISED IN AN INTENSIVE SYNBIOTIC SYSTEM

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Introduction

The enhancement of shrimp growth and immunity represents a crucial aspect of the prevention of economic losses. Consequently, bioproducts and microbial cells are regarded as superior alternatives for the management of animal health, facilitating accelerated growth and enhanced disease resistance. *Saccharomyces cerevisiae* (either in single-cell protein or whole cell form) has been reported to contain various immunostimulatory compounds such as β -glucans, nucleic acids, nucleotides, and chitin, as well as mannan oligosaccharides (MOS) (Navarrete and Tovar-Ramrez 2014). Nucleotides are low molecular weight intracellular and immunomodulatory compounds that serve as the basic building blocks of DNA and RNA. They play a crucial role in various physiological processes in living organisms, including cell division, modulation of cell growth, regulation of the immune system, and maintenance of gut health (Biswas et al. 2012). Therefore, this study evaluated different levels of nucleotide addition on growth performance, ammonia and nitrite stress, and return on investment (ROI) of Pacific white shrimp, *Penaeus vannamei*, raised in an intensive synbiotic system.

Materials 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*. This diet was designed to be isoproteic, 360 g kg⁻¹, and isoenergetic, 4400 kcal kg⁻¹. Furthermore, three test diets were formulated with the inclusion of a commercial nucleotide source to replace wheat flour in the control diet at levels of 75, 150, and 300 mg nucleotides per kg feed⁻¹, designated N75, N150, and N300, respectively. The commercial nucleotide source consists of 15% RNA (nucleotides) from the yeast *Saccharomyces cerevisiae*, 50% crude protein, 15% ash, and 8% water (Biotide Extra, Biorigin, São Paulo, Brazil). The study was conducted over 60 days at the Laboratorio de Carcinicultura (LACAR), of the Departamento de Pesca e Aquicultura (DEPAq) at the Universidade Federal Rural de Pernambuco (UFRPE), Brazil. The system was prepared in twelve fiberglass tanks filled with 600 L of seawater (32 g L⁻¹) filtered to 250 μ m and chlorinated with 30 ppm of active chlorine using sodium hypochlorite. During the feeding trial, the shrimps were fed three times daily (8:00 am, 01:00 pm, and 5:00 pm) with the experimental diets detailed in Table 1. Initially, the daily feeding rate was 8.0% of body weight, gradually reduced to 4.7% over 60 days. The feeding rate was adjusted daily based on estimated shrimp feed consumption and mortality (Van Wyk et al. 1999). Shrimp weight ($n = 20$) was monitored every 10 days to determine growth and to adjust the amount of feed amount. At the 30 days and at the end of the experimental period, all shrimp were counted and final weight, feed conversion ratio (FCR), survival and yield were determined. At the end of the growth trial, a total of 30 shrimps ($n = 10$ per replicate) were randomly collected from each experimental treatment and subjected to a stress test for ammonia nitrogen (NH₃-N) levels in the rearing water (Oliveira et al. 2022; Pimentel et al. 2022). Subsequently, 24 shrimps ($n = 8$ per replicate) were used for the NO₂-N stress test. In each test, experimental units containing 10 L of water (salinity 30 ± 0.6 g L⁻¹, water temperature 28 ± 0.4 °C, and pH 7.5 ± 0.2) were used. The NH₃-N concentration was achieved by applying a stock solution of 10 g L⁻¹ of NH₄Cl (PA) and the NO₂-N concentration was achieved by applying a stock solution of 49.25 g L⁻¹ NaNO₂ (PA). Nucleotide costs and feed processing were considered alongside shrimp performance to determine the return on investment (ROI) according to Phillips and Phillips (2019). Feed production costs in this trial included feed at US\$1.03 per kilogram, nucleotide at US\$6.50 per kilogram, and shrimp priced at US\$3.50 per kilogram, with an estimated production for 1 ha. ROI = (Net benefit with nucleotide addition – Net benefit in diet control) / difference in total expenses Nucleotide $\times$ control.

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Results

Significant differences between treatments were found for final weight, growth (g week^{-1}), yield kg m^{-3} , with higher values observed in the nucleotide addition treatments. FCR was lower in all nucleotide treatments than in the control. Significant differences between treatments were also found for survival at day 60. With regard to ammonia nitrogen stress tests, the survival of *P. vannamei* with nucleotide addition was between 77.8 and 80.6% and the control 75%, although without significant differences. Similarly, for nitrite nitrogen stress tests, survival was between 79.2 and 83.3% for nucleotide treatments and 70.8% for control, although without significant differences. For the dietary nucleotide treatments, the income from shrimp sales minus the cost of feed ranged from US\$ 29,244 ha^{-1} to US\$ 30,542 ha^{-1} for N75 and N300, respectively. Alterations such as an increase in villus height (significant findings obtained in this study and not discussed here) result in an expansion of the surface area available for nutrient absorption, which can consequently increase the efficiency of digestion and nutrient absorption in aquatic animals. In this study, it was observed that the height of villi, gut folds and enterocytes was significantly higher (data not discussed) in the nucleotide addition treatments than in the control treatment, which may indicate that the shrimp fed with nucleotide had a higher nutrient absorption capacity, reflecting in production performance and consequently ROI.

Conclusion

The addition of nucleotides (75, 150, and 300 mg kg feed^{-1}) to the diet of *P. vannamei* reared in an intensive synbiotic system improved growth, survival, and yield compared to the control treatment. The addition also improved gut health (FH—fold height; VH—villus height; and EH—enterocyte height) and return on investment. The most recommended dose is 150 $\text{mg nucleotide feed kg}^{-1}$, as it gave the highest ROI.

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REFORMULATION OF ATLANTIC SALMON (*Salmo salar*) FEEDS USING ALTERNATIVE AND CIRCULAR INGREDIENTS WITHOUT COMPROMISING PERFORMANCE OR PRODUCT QUALITY

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Introduction

Sustainable growth of Atlantic salmon aquaculture requires increasing production to meet rising global demand while maintaining the use of marine resources such as fishmeal (FM) and fish oil (FO) within sustainable limits. This requires the development of alternative feed ingredients that support production without increasing reliance on finite marine resources. Emerging alternatives, including microalgae-derived oils, insect-based ingredients, and circular by-products, have been investigated individually; however, their combined use within complete feed formulations at commercial scale remains insufficiently evaluated. The Millennial Salmon project (Research Council of Norway #319987) aimed to develop next-generation aquafeeds integrating these alternative resources and to assess their biological performance and product quality under commercial like farming conditions. The present study represents the final validation stage of the project, in which fully reformulated diets were tested in a pilot-scale sea-cage trial.

Material and Methods

A feeding trial was conducted in 5x5m sea cages from June to October 2025 at Mowi's Field Trials Station (Averøy, Norway). Atlantic salmon were grown from approximately 3kg to 5kg mean body weight. Four experimental diets were tested in quadruplicate sea cages:

- **Control diet:** conventional commercial formulation
- **Millennial Salmon diet:** optimised low-carbon formulation incorporating project-validated alternative ingredients, including insect meal and oil and microalgae-derived DHA-rich oil
- **Low Marine diet:** high reduction of marine-derived ingredients
- **Circular diet:** high inclusion of circular raw materials (poultry by-products, salmon oil, insect meal and oil, microalgae-derived DHA-rich oil)

The diets were formulated to meet the known nutritional requirements of Atlantic salmon and represent distinct sustainability strategies. Growth performance, feed utilization, and apparent digestibility coefficients (ADC) were evaluated alongside whole-body composition and fatty acid profiles. Product quality traits, fish health and welfare indicators, and oxidative status were also assessed, including lipid peroxidation and antioxidant capacity in liver and intestinal tissues. In addition, the carbon footprint (in kg CO₂eq. calculated with the IPCC 2021 100-year GWP with land-use change methodology) of the feed concepts per kg feed and per FCR was evaluated using life cycle assessment (LCA) with economic allocation.

Results and Discussion

All alternative diets resulted in comparable growth and production performance, with no significant differences observed in final body weight, specific growth rate (SGR), feed conversion ratio (FCR), or slaughter yield relative to the Control diet. ADC of protein, fat, and energy remained high across treatments, indicating efficient utilization of the alternative and circular raw materials. Dietary lipid composition influenced whole-body fatty acid profiles, reflecting the use of alternative lipid sources. However, these compositional differences were not associated with changes in fillet quality, as no differences were observed in pigmentation or instrumental colour (Minolta measurements), suggesting preservation of key product quality traits under the tested formulations. No adverse effects on fish health or welfare indicators were detected. Fish fed the Millennial Salmon diet exhibited improved fin condition, reduced skin lesion prevalence, and a lower incidence of cataract formation compared to the Control ($p < 0.05$). Liver lipid peroxidation increased progressively with decreasing inclusion of marine ingredients, with fish fed the Low Marine diet showing significantly higher malondialdehyde (MDA) concentrations than other treatments. These changes were not accompanied by structural alterations in liver tissue, as histological examination (**Figure 1**) revealed no pathological findings across dietary groups. The observed responses were therefore interpreted as adaptive physiological adjustments. To complement the biological and product quality assessments, the carbon footprint of the feed concepts was evaluated using LCA. The Circular diet exhibited the lowest carbon footprint

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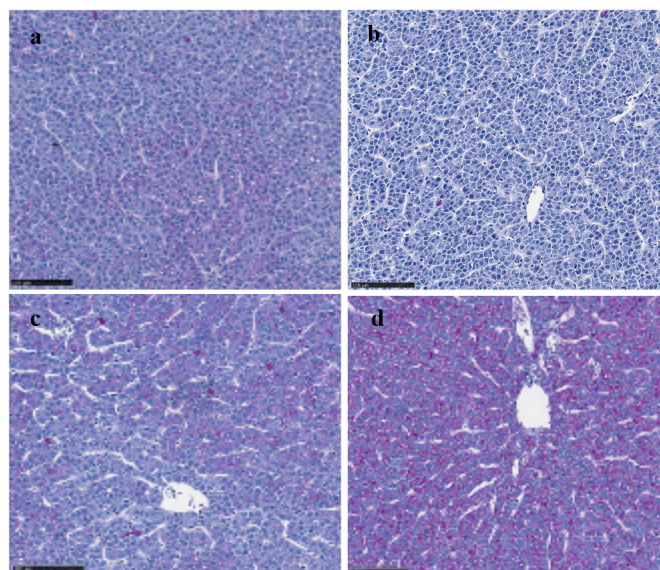


Figure 1 Representative liver histology of Atlantic salmon fed Control (a), Millennial Salmon (b), Circular (c), and Low Marine (d) diets, showing adaptive metabolic responses.

(kg CO₂-eq), both per unit of feed and when expressed relative to FCR. The Control diet showed the highest footprint per kg of feed. When expressed per unit of production (FCR-adjusted), the Low Marine diet exhibited a slightly higher carbon footprint than the Control, reflecting differences in feed efficiency among treatments.

Conclusions

The present study indicates that the integrated inclusion of microalgae, insect-derived ingredients, and circular by-products can substantially reduce reliance on marine resources in Atlantic salmon feeds without adversely affecting growth performance, welfare indicators, or product quality under the tested conditions. The results further suggest that dietary lipid composition and oxidative stability remain important considerations in the formulation of low-marine feed concepts, particularly in diets enriched with alternative lipid sources. From an environmental perspective, LCA showed that feed formulations incorporating *Schizochytrium* sp. biomass and insect-derived ingredients may reduce the carbon footprint of salmon feed, depending on formulation strategy and system boundaries. These findings highlight the importance of evaluating environmental performance at the level of complete feed formulations and production efficiency, including FCR, rather than at the ingredient level alone. Further analyses of oxidative status and antioxidant responses in additional tissues are ongoing and will provide a more comprehensive understanding of the physiological implications of these feed formulations.

MICROBIAL COMMUNITIES IN RECIRCULATING AQUACULTURE SYSTEMS: EXPLORING THE ROLE OF DIET AND NUTRIENT DYNAMICS ON ASSEMBLY MECHANISMS

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Background and approach

Recirculating aquaculture systems (RAS) are increasingly adopted in marine aquaculture due to their sustainability and efficiency. Central to their performance are microbial communities, which play vital roles in nutrient cycling, water quality maintenance, and ultimately, fish health and welfare. While nutrient dynamics are well-recognized drivers of microbial community structure, the influence of diet—particularly via fish-derived nutrient excretion—remains underexplored, especially in the context of novel aquafeed ingredients (Syropoulou et al., 2025). As the aquaculture industry transitions toward sustainable feed alternatives, it becomes essential to understand their impacts on microbial ecology within RAS. Microbial assembly processes indicate how a community of microorganisms comes together and forms a certain composition, and it is governed by a dynamic interplay between deterministic (predictable, niche-based) and stochastic (random, neutral) processes (Li et al. 2022). These forces determine which microorganisms colonize, survive, and interact within a specific environment, and can be largely affected by nutrient presence.

In this study, we investigated how diet, through fish excretion and water nutrient dynamics, influences microbial community structure and assembly processes across key RAS compartments: water, biomedica, and fish skin. For this purpose, we collected samples from two experimental trials with juvenile European seabass, reared under controlled conditions in marine RAS, and fed either a plant-based or chitin-based diet over two six-week trials. We performed biweekly samplings across the three niches and analysed nutrients in the water (Chemical and biological oxygen demand, total nitrogen, bacterial activity) along with 16S rRNA gene sequencing to characterize the microbial communities.

Results and Discussion

Our findings revealed significant effects of both diet and time on the alpha diversity of water-associated microbial communities, whereas fish skin communities were primarily shaped by temporal dynamics. In terms of beta diversity, diet significantly influenced the phylogenetic similarity (measured as Weighted Unifrac distance) in water and skin microbiota (Figure 1B), but not in biomedica. Levels of dissolved carbon and nitrogen in the water explained up to 50% of the variation (Figure 1A), showing the nutrients are essential shaping the microbial communities. Notably, fish skin and water shared the greatest number of taxa and exhibited similar taxonomic profiles. Nutrient gradient analysis indicated that carbon was the primary driver of microbial turnover, followed by nitrogen. Community assembly processes varied by niche: stochastic processes (drift) predominantly shaped water and skin microbiota, while deterministic processes (selection) played a larger role in biomedica. The type of diet modulated these dynamics; drift dominated in fish fed chitin-based diets, whereas homogeneous dispersal was more prevalent under plant-based diets in water and skin. In biomedica, dispersal remained a key factor regardless of diet, with selection contributing significantly.

In summary, our results demonstrate that dietary inputs, via fish excreta, can substantially influence microbial communities in water and on fish skin, with carbon gradients acting as major ecological drivers. Although diet had a measurable impact on community structure, its influence on microbial assembly mechanisms was more niche-dependent and less pronounced. These findings highlight the importance of considering feed formulation not only for fish nutrition but also for optimizing microbial dynamics in RAS ecosystems.

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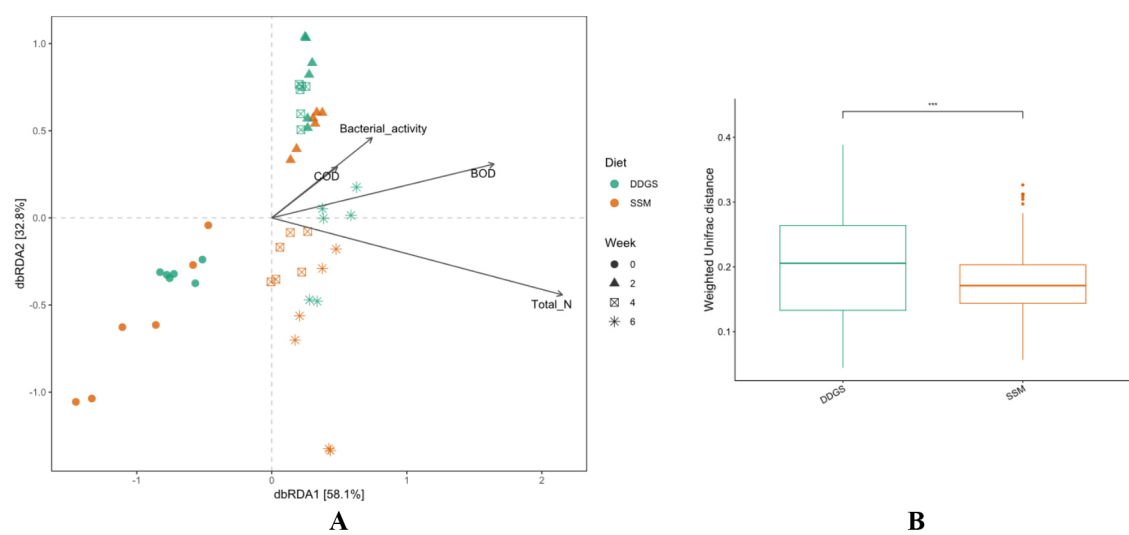


Figure 1. A. Distance-based (Bray Curtis) redundancy analysis (dbRDA) on water microbial communities over the six week experimental period. Vectors indicate the weight and direction of the different water nutrient parameters; COD: Chemical Oxygen Demand; BOD; Biological Oxygen Demand. The dbRDA axes describe the percentage of the fitted or total variation explained by each axis while being constrained to account for group differences. B. Weighted Unifrac distance showing within group variation in the plant-based (DDGS) and the chitin-based (SSM) diets. Stars indicate significance $P<0.001$.

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COMPARATIVE HYPOXIA TOLERANCE OF PIKEPERCH (*Sander lucioperca*) AND ITS HYBRID (*S. lucioperca* × *S. volgensis*)

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Introduction

Fish hybrids play a significant role in modern aquaculture by offering the potential for improved growth rates, disease resistance, and adaptability to various environmental conditions (Uno et al., 2025; Marnis et al., 2026). Hybridization, especially between closely related species such as pikeperch (*Sander lucioperca*) and Volga pikeperch (*Sander volgensis*), has attracted interest for its potential to enhance production characteristics and address specific challenges in aquaculture (Stanivuk et al., 2026). The hybridization of the two species aims to combine the advantageous traits of the economically valuable pikeperch and the Volga pikeperch. The objective of the present study was to compare the oxygen tolerance of pikeperch and its hybrid.

Materials and Methods

Pikeperch (*S. lucioperca*) and hybrid (*S. lucioperca* × *S. volgensis*) fingerlings were maintained under controlled laboratory conditions. Both pikeperch and hybrid fish were obtained through artificial propagation. During the first two months after hatching, the fish were fed live prey before being gradually weaned onto a formulated artificial diet. Feeding was discontinued 24 h prior to the hypoxia trials. Experimental groups consisted of five pure pikeperch and five hybrid individuals, with three independent replicates established for each treatment. Fish were randomly allocated to cylindrical experimental tanks (Table 1). Each trial was performed in a cylindrical vessel with a diameter of 15 cm and a total volume of 2000 mL, containing 1800 mL of water. Throughout the experiment, the water temperature was maintained at 20.5 °C.

The endpoint of the hypoxia test was defined as the loss of equilibrium, indicated by the fish turning upside down and remaining immobile for at least 5 s. At this point, individuals were immediately transferred to oxygenated water in accordance with the protocol described by Müller et al. (2006). For each fish, genotype, time to loss of equilibrium (s), and dissolved oxygen concentration (mg L⁻¹) were recorded using an OxyGuard Handy dissolved oxygen meter (Gamma®, Birkerød, Denmark). Following the removal of each fish, approximately one-tenth of the water volume was simultaneously withdrawn to prevent an artificial increase in the relative oxygen concentration within the experimental tank. No mortality occurred during the experimental period.

Results

The independent-samples t-test revealed no statistically significant difference between the two groups ($t(28) = -0.99$, $p = 0.165$, Cohen's $d = 0.36$).

Discussion

The present study demonstrated that pikeperch and hybrid juveniles of approximately 1 g body weight exhibited similar tolerance to progressive hypoxia, with no significant differences in either the time to loss of equilibrium or the dissolved oxygen concentration at the endpoint. Müller et al. (2006) likewise reported similar critical dissolved oxygen concentrations in approximately 9 g pikeperch and hybrids, although pure pikeperch tolerated hypoxia for a significantly longer time. In contrast, no difference in exposure time was observed in the present study, suggesting that genotype-related differences may not yet be expressed at this early developmental stage. The discrepancy between studies is likely related to differences in fish size, as ontogenetic changes can substantially influence hypoxia tolerance. The small effect size observed in the present study (Cohen's $d = 0.36$) further indicates only minor biological differences between the two genotypes. Further studies including larger fish and additional developmental stages are needed to clarify how hybridization influences hypoxia tolerance during ontogeny.

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Table 1. Body weight and oxygen tolerance characteristics of pikeperch and hybrid fish. Values are presented as mean $\pm$ SD. Same superscript letters indicate no significant differences between groups ($p > 0.05$). Oxygen tolerance is expressed as the time to loss of equilibrium and the corresponding maximum and minimum dissolved oxygen concentrations.

fish	Body weight (g)	O ₂ tolerance		
		Time (min)	Maximum level (mg L ⁻¹)	Minimum level (mg L ⁻¹)
pikeperch	1.03 $\pm$ 0.11 a	98.6 $\pm$ 20.8 a	0.09	0.47
hybrid	1.04 $\pm$ 0.08 a	105.0 $\pm$ 13.4 a	0.08	0.36

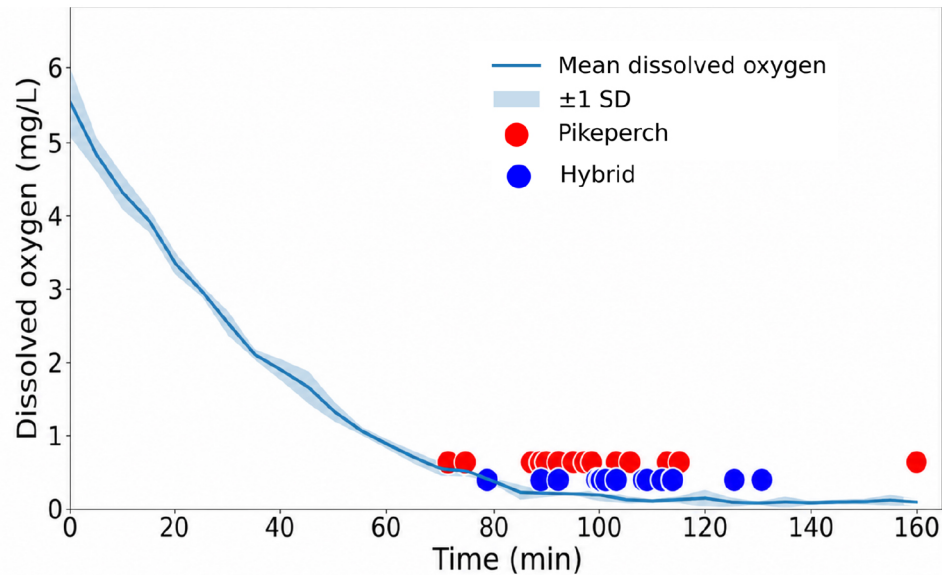


Figure 1. Mean dissolved oxygen concentration (solid line $\pm$ SD) during the hypoxia challenge and dissolved oxygen concentrations at loss of equilibrium (LOE) in individual pikeperch (red circles) and hybrid (blue circles).

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ENVIRONMENTAL ENRICHMENT DOES NOT COMPROMISE RAINBOW TROUT (*Oncorhynchus mykiss*) GROWTH: POTENTIAL WELFARE BENEFITS OF NON-PHYSICAL ENRICHMENT

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Introduction

As aquaculture production becomes more intensive, improving the welfare of cultured fish has become an important research goal. Environmental enrichment (EE) can provide additional stimulation that supports physiological, behavioral, and psychological needs of fish (Arechavala-Lopez et al. 2022). Previous studies have shown positive effects of EE on salmonid growth and welfare, particularly during early life stages (Kientz & Barnes 2016; Jones et al. 2019). However, the effects of EE on larger fish under production-like conditions remain less studied. Physical EE can increase environmental complexity, but practical limitations related to cleaning and management may restrict its use in aquaculture (Gerber et al. 2015). Therefore, non-physical EE methods may provide practical alternatives for recirculating aquaculture systems (RAS). This study investigated the effects of physical and non-physical EE on growth and welfare of 1+ rainbow trout (*Oncorhynchus mykiss*) reared in RAS.

Methods and materials

A total of 515 rainbow trout were randomly distributed among 12 tanks. The experimental unit was the tank, with three replicate tanks per treatment. Fish were exposed to four treatments: unenriched control, physical EE consisting of suspended plastic sticks, and two non-physical EE treatments consisting of changing water flow direction and colored light variation. Growth was monitored using tank-level mean weight, specific growth rate (SGR), and apparent feed conversion ratio (aFCR). At the end of the experiment, individual fish were assessed using the Laksvel protocol (Nilsson et al. 2025), including external injuries, fin condition, injury severity, and active lesions. Individual body length and weight were measured to calculate Fulton's condition factor (K).

Results

Rainbow trout growth was not significantly affected by EE treatment, and no significant differences were observed in injury levels or severity. However, fish exposed to changing water flow and colored light showed trends towards higher final weight, better SGR, and improved aFCR compared with control and physical EE treatments. Almost all fish had some external injuries at the end of the experiment, with caudal fin damage and eye opacity being the most common findings. The physical EE treatment showed a tendency towards increased eye injuries and less severe injuries, whereas non-physical EE treatments showed lower total injury rates compared with the control and physical EE treatment. These effects were not statistically significant after correction for multiple comparisons.

Discussion

The results indicate that EE did not negatively affect production performance or external welfare indicators. Flow and colored light EE showed trends towards improved growth and feed utilisation compared with control and physical EE treatments, although differences were not statistically significant. The lack of significant effects may be related to the short experimental period and larger fish size compared with previous studies, which have mainly focused on early life stages (Näslund & Johnsson 2016). The effects of EE may depend on species, life stage, and exposure duration, and welfare benefits in larger fish may occur through mechanisms other than growth enhancement (White et al. 2018). Physical EE may present practical challenges in aquaculture if structures interfere with feeding or tank management, whereas non-physical EE methods may provide feasible alternatives for increasing environmental complexity in RAS (Gerber et al. 2015). Further studies should investigate behavioural and physiological responses to EE and assess long-term effects under production conditions.

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JUVENILE QUALITY IN FISH HATCHERIES: A SISYPHIAN CHALLENGE?

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Introduction

Juvenile quality is a major determinant of productivity, fish welfare, and economic sustainability in Mediterranean aquaculture. Among quality-related traits, skeletal abnormalities remain one of the most persistent challenges in marine fish hatcheries, affecting biological performance, morphology, and commercial value of the final product (Boglione et al., 2013). Since the early development of Mediterranean aquaculture, significant progress has been achieved through improvements in larval rearing protocols, selective breeding programs, and morphological quality control. As a result, the prevalence of malformed fish has substantially decreased during the last decades. Nevertheless, important variability among hatcheries, production cycles, and fish populations still occurs, while novel deformity phenotypes continue to emerge under standard commercial conditions (Kourkouta et al., 2022; Kourkouta et al., 2025). In this presentation, emphasis will be placed on the major factors contributing to the quality downgrading of reared fish larvae, the critical ontogenetic stages involved, and the proposed management practices for addressing the problem in hatcheries. In addition, recent research findings will be presented regarding the assessment of the severity of skeletal deformities at the production level.

Anatomical and ontogenetic differentiation of skeletal abnormalities

Skeletal abnormalities differ considerably in terms of affected anatomical structures, developmental timing, and biological severity (Boglione et al., 2013). Most abnormalities develop during skeletogenesis up to metamorphosis, whereas others, such as haemal lordosis and Ray-Resorption Syndrome (RSS), may appear after completion of skeletal development on initially normal skeletal elements (Sfakianakis et al., 2006; Kourkouta et al., 2025). Each deformity type is characterized by a specific critical ontogenetic window associated with the developmental pattern of the affected skeletal structures (Koumoundouros, 2010). This ontogenetic specificity highlights the strong developmental plasticity of marine fish larvae and partly explains the difficulty in defining universally optimal rearing conditions for normal skeletal development.

Causative factors and hatchery variability

The development of skeletal abnormalities is multifactorial, involving nutritional, abiotic, and genetic determinants (Boglione et al., 2013; Fragkouli et al., 2018). Despite extensive research efforts, the identification of widely used optimal environmental and nutritional conditions remains challenging because larval physiological requirements vary substantially throughout ontogeny (Mazurais et al. 2009, Georgakopoulou et al. 2010). In commercial hatcheries, skeletal abnormality incidence is additionally characterized by high variability both among production batches and among populations within the same production cycle, suggesting that small deviations in complex production protocols may substantially influence larval skeletal quality (Kourkouta et al., 2022).

Emerging management approaches and future perspectives

Recent findings are progressively changing the traditional perception that skeletal abnormalities represent irreversible developmental defects. Experimental and commercial-scale studies have demonstrated that some abnormalities, including haemal lordosis and upper jaw deformities, may partially recover during on-growing (Fragkouli et al., 2019, 2022; Geladakis et al., 2025). In parallel, advances in morphometric tools provide new opportunities for early diagnosis and severity-based classification of skeletal abnormalities (Geladakis et al., 2025). Recent nutritional approaches aiming to enhance skeletal resilience and mineralization capacity further support the development of preventive management strategies (Printzi et al., 2024). At the production level, integration of automation systems, benchmarking approaches, certified raw materials, and large-scale production data may improve protocol standardization and reduce within-hatchery variability. Future improvements in skeletal quality management will likely depend on combining precision hatchery practices with selective breeding programs incorporating morphological quality traits.

Conclusions

Current evidence indicates that skeletal abnormalities should be viewed as dynamic phenotypes influenced by developmental plasticity, recovery capacity, and hatchery management. Integrating precision phenotyping, resilience-oriented nutrition, and standardized production protocols may substantially improve skeletal quality, fish welfare, and production sustainability in Mediterranean aquaculture.

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GRAM-NEGATIVE BACTERIAL BIOMASS IN THE DIET ENHANCES FEED INTAKE AND IMMUNE READINESS IN ATLANTIC SALMON DURING SMOLTIFICATION AND TRANSFER

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Introduction

The continued expansion of aquaculture requires sustainable protein sources beyond fishmeal, as pressure on wild fisheries and rising costs limit its long-term viability (FAO, 2018). Bacterial meal (BM), a single-cell protein produced from bacteria grown on non-food substrates, emerges as a promising alternative due to its high protein content, favourable amino acid profile, and low land and water requirements (Pesante et al., 2022), yielding good growth and health in farmed fish, including salmon (Aas et al., 2006). BM also serves as a source of postbiotics, nonliving microbial components, whereas Gram-negative bacterial meals have shown dose-dependent improvements in growth performance, microbiota diversity, and inflammatory regulation in fish (Wang et al., 2023). In the current study, graded dietary inclusion of a Gram-negative bacterial biomass produced from agricultural sidestreams was evaluated for its effects on Atlantic salmon smolt performance, smoltification success, and key health and welfare indicators.

Materials and Methods

The feeding trial was conducted at the Nofima Research Station for Sustainable Aquaculture (Sunndalsøra, Norway) using four diets containing 0, 2.5, 5, or 10% marine BM, each fed to triplicate groups of Atlantic salmon parr (~80 g initial body weight). Fish were reared for 9.5 weeks in 12 tanks under controlled freshwater/seawater conditions and continuously fed using automatic feeders, with feed intake and mortalities recorded daily. Growth performance, feed utilisation, smoltification capacity, and biometry were assessed. Moreover, blood biochemistry, hindgut histology, and enzyme activities were analysed. Head kidney transcriptomics was performed using Nofima's 44k Atlantic salmon microarray (Salgeno - 2) using the STARS bioinformatics platform (Krasnov et al., 2011).

Results and Discussion

Feed intake (% BW/day) increased stepwise with dietary BM inclusion, with statistically significant differences observed between the control and 10% BM after seawater transfer, and overall, between 5% BM and the control. Feed intake correlated with growth, and fish in all BM treatments were numerically larger at the end of the trial, although the growth differences were not statistically significant. Relative liver weight (HSI) was significantly higher in the BM-fed fish, which may be linked to immune stimulation by bacterial cell wall components rather than to high-fat-driven liver enlargement. No differences in mortality were observed across treatments. In terms of population structure, the BM groups showed significant differences in size distribution (higher kurtosis at 5–10% BM), consistent with a stronger clustering around one body size rather than evenly spread “small-to-large” categories, an observation that is relevant given that dominance hierarchies can influence growth dispersion in salmon populations (Soares et al., 2019). This aligns with the broader theory that bacterial meals/postbiotics can affect behaviour and stress responsiveness *via* microbiota-born signals (e.g., Knobloch et al., 2022). Blood biochemistry showed the lowest cholesterol at 10% BM and the highest in the control group, whereas triglycerides tended to show the opposite trend (higher at 5–10% BM). Hematocrit and the remaining blood parameters were unchanged. Histology of the distal intestine (AI-based Aiforia® analysis) revealed no signs of enteritis in any group, and the distal intestine layers (including lamina propria and submucosa) showed no inflammatory thickening. Nevertheless, distal enterocyte height (mucosa width) was significantly lower in fish fed 2.5% BM compared with the control. Vacuolisation was medium-to-high in all groups (numerically higher in BM fish), interpreted as an “active” distal intestine consistent with lipid digestion/absorption. Mucous cell numbers decreased slightly in BM groups, with a statistically significant reduction in large mixed-mucin (blue) mucous cells at 2.5% BM. Intraepithelial lymphocytes were significantly reduced at 10% BM (and their localisation shifted toward the mucosal surface), suggesting immunomodulation without an enteritis phenotype. The eosinophilic granular cells varied, being highest at 2.5% BM. These outcomes are compatible with the

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mixed performance evidence for bacterial protein meals in salmonids, with some studies reporting improved growth and nutrient utilisation even at high inclusion levels (e.g., up to 36% bacterial protein meal; Aas et al., 2006), while other functional feed strategies have shown a shift in microbiota composition without growth gains (Baumgärtner et al., 2022). Overall, the trial supports the inclusion of BM (2.5-10%) as gut-safe under these conditions, with functional effects on feed intake and immune-related gut cell profiles, while highlighting that the “optimal” inclusion window likely depends on balancing immune stimulation against potential metabolic trade-offs.

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FROM SCIENCE TO INSPECTION: BUILDING CAPACITY FOR FISH WELFARE ASSESSMENT THROUGH EURCAW-AQUA

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Introduction

Effective assessment of fish welfare during official controls requires competent authorities and veterinary inspectors to translate scientific knowledge into practice. However, the diversity of farmed fish species and production systems, limited species-specific guidance, and differences in national practices present challenges for consistent welfare assessment across the EU Member States. To support the implementation of high welfare standards in aquaculture, operating under the European Commission, the European Reference Centre for the Welfare of Aquatic Animals (EURCAW-Aqua) conducts species-specific research on welfare in various aquaculture systems and facilitates dissemination of available scientific knowledge to national competent authorities. As part of this role, EURCAW-Aqua partner, the Biology Centre of the Czech Academy of Sciences (BCAS), developed and delivered a series of webinars addressing key aspects of welfare assessment of farmed common carp (*Cyprinus Carpio*) and rainbow trout (*Oncorhynchus mykiss*).

Materials and Methods

Four webinars were delivered between 2024 and 2026 for veterinary inspectors and representatives of national competent authorities involved in official controls of fish welfare. Training content combined current scientific evidence, EU regulatory requirements, expert knowledge and practical experience. The agenda was structured to ensure a balance between theoretical input and practical relevance, incorporating presentations from international experts, interactive discussions, and participant engagement tools such as Slido. The main topics included the use of operational welfare indicators (OWIs), welfare assessment methodologies, and welfare risks associated with handling and transport. International experts contributed scientific and practical perspectives, while interactive tools facilitated real-time participation and knowledge exchange. The delivery of sessions was continuously refined based on participant feedback and experience from previous sessions, particularly through the incorporation of field-based case experiences in response to participants' requests. Webinar attendance, geographical representation, participant feedback and interactive responses were used to evaluate the reach and perceived relevance of the training and to identify recurring topics and challenges raised by competent authorities.

Results

The four webinars reached participants from 18 EU Member States, with attendance ranging from 30 to 69 participants per webinar. Invited experts represented ten European countries, providing perspectives from different aquaculture systems, regulatory contexts and areas of fish welfare expertise. Interactive quizzes were incorporated into three webinars and revealed important gaps in the practical assessment of fish welfare. Participants identified the lack of clear guidelines and difficulties in assessing fish behaviour as major challenges, despite behavioural observations being among the most commonly used indicators in practice. For fish transport, participants expressed a need for more specific guidance on water-quality parameters. Notably, among respondents during the webinar on trout transport, 60% reported having no access to clear protocols or guidelines for welfare assessment, while only 10% reported access to detailed and practical guidance. In the post-webinar questionnaires, on average 96% respondents expressed overall satisfaction or high satisfaction with the webinars and 83% rated the training as relevant or highly relevant to their professional activities. Across the webinar series, recurring topics included the practical application of operational welfare indicators (OWIs), welfare assessment during handling and transport, methodological approaches to welfare monitoring, and differences in interpretation and implementation of welfare regulations between different EU countries.

Conclusion

The EURCAW-Aqua experience demonstrates that capacity building can function as more than a one-way transfer of knowledge. The interactive training provided a platform for translating scientific evidence into practice, while also drawing on participants' experiences, questions and feedback to identify knowledge gaps and areas requiring further scientific guidance or harmonization. Continued dialogue among researchers, veterinary inspectors and competent authorities can therefore support more consistent approaches to fish welfare assessment and official controls across the EU Member States.

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EVALUATION OF GROWTH ENHANCEMENT AND TOXICITY OF ASTAXANTHIN-LOADED SOLID LIPID NANOPARTICLES IN *Artemia* sp.

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Introduction

Astaxanthin (ASTX) is a carotenoid extensively used in aquaculture because of its antioxidant activity and pigmentation properties. However, its application is limited by poor water solubility and susceptibility to degradation under environmental conditions. Solid lipid nanoparticles (SLNs) have emerged as promising carriers capable of improving the stability and delivery of lipophilic compounds. This study aimed to develop ASTX-loaded SLNs and evaluate their physicochemical characteristics, toxicity, and biological effects in *Artemia* sp.

Materials and Methods

SLNs composed of stearic acid and Tween 20 were synthesized and loaded with ASTX. Nanoparticles were characterized by transmission electron microscopy (TEM), dynamic light scattering (DLS), zeta potential analysis, and Fourier-transform infrared spectroscopy (FTIR). Toxicity was assessed through acute exposure assays to determine median lethal concentrations (LC50). Growth performance was evaluated by measuring body length and width, while oxidative status and metabolic activity were determined through reactive oxygen species (ROS) production and electron transport system (ETS) activity, respectively.

Results

The developed formulation achieved an ASTX concentration of 0.47 mg mL⁻¹ and an encapsulation efficiency of 87.3%. TEM analysis revealed nanoparticles with an average diameter of 45.0 ± 1.4 nm, whereas DLS and zeta potential measurements indicated satisfactory colloidal stability. FTIR spectra confirmed the successful incorporation of ASTX into the lipid matrix. Acute toxicity assays resulted in LC50 values of 0.21 mg mL⁻¹ for unloaded SLNs and 0.13 mg mL⁻¹ for ASTX-loaded SLNs. Despite the increased toxicity associated with the encapsulated formulation, exposure to a sublethal concentration of 0.06 mg ASTX mL⁻¹ significantly enhanced both body length and width in *Artemia* compared with the control group. No significant differences were observed in ETS activity among treatments. In contrast, ROS production was influenced by nanoparticle composition, with unloaded SLNs inducing a pro-oxidant response and ASTX-loaded SLNs reducing oxidative stress at intermediate concentrations.

Conclusion

ASTX-loaded SLNs improved growth performance and contributed to oxidative balance in *Artemia* sp. when applied at concentrations below 0.12 mg ASTX mL⁻¹. These findings demonstrate the potential of lipid-based nanoformulations as innovative delivery systems for bioactive compounds in aquaculture, supporting their future application in nutritional and functional feeding strategies.

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OPTIMIZING STOCKING DENSITY OF PIKEPERCH (*Sander lucioperca*) GROW-OUT CULTURE WITHIN IN-POND RACEWAY SYSTEM

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Introduction

Pikeperch (*Sander lucioperca*) is a species of growing importance in European aquaculture due to its high market value (Polícar et al., 2019) and suitability for intensive production systems. The In-Pond Raceway System (IPRS) represents a promising semi-intensive approach combining advantages of pond culture and controlled rearing conditions (Nagy et al., 2022, Kučera et al., 2025). However to be able to further incorporate the IPRS in European inland aquaculture practices, optimization is necessary to ensure feasibility and effective operation of this production system. Therefore, optimization of stocking density, one of the most influential parameters (Ljubobratović et al., 2016, Kozłowski and Piotrowska 2024) is essential. This study aimed to evaluate the effects of different stocking densities on growth performance, feed utilization, and physiological status of grow-out sized pikeperch reared in IPRS.

Material and methods

The experiment was conducted over 24 weeks (168 days) from May to September in an IPRS installed in a 0.35 ha pond. A total of 2,304 pikeperch (initial body weight ~160 g) were randomly distributed into three experimental groups: G20 (20 kg.tank⁻¹; 2.78 kg.m⁻³), G40 (40 kg.tank⁻¹; 5.56 kg.m⁻³), and G60 (60 kg.tank⁻¹; 8.33 kg.m⁻³), each in triplicate. Fish were fed a commercial diet Le Gouessant Percid Grower Flot 5, daily feeding ratio was set between 0.8-1.5% of biomass. Growth, feed conversion ratio (FCR), survival rate (SR), and condition factor (CF) were evaluated. Blood sampling was carried out to assess biochemical profile, haematology and oxidative stress. Dissection of euthanized fish was done to determine organosomatic indices and to sample tissues for oxidative stress analysis. Water quality remained within suitable ranges for pikeperch culture, however with relatively low average temperature (16.84 ± 3.41°C) and moderate dissolved oxygen saturation (79.93 ± 6.08%).

Results and discussion

Growth was comparable among all treatments, indicating that stocking density up to 8.33 kg.m⁻³ did not negatively affect growth. In contrast, FCR and SR improved significantly with increasing density, with the most favourable values observed in G60 and comparable to RAS based rearing (Pěnka et al., 2024, Polícar et al., 2016). CF decreased slightly at higher densities, suggesting a leaner body composition. Visceral fat index (VFI) increased markedly during the experiment, with no significant differences among groups, with results similar to published studies (Polícar et al., 2024; Kučera et al., 2025). Biochemical analysis revealed increased hepatic enzyme activity and elevated protein fractions in G40 and G60, indicating intensified metabolic activity and adaptive physiological responses consistent with grow-out. Elevated triglycerides and lipase activity further supported enhanced lipid metabolism at higher densities. Oxidative stress responses were tissue-specific. While mucus showed slightly increased lipid peroxidation and antioxidant activity at higher densities, plasma and liver parameters indicated effective antioxidant defence without systemic oxidative damage. Haematological parameters, particularly increased haematocrit and mean corpuscular volume (MCV) in G40 and G60, suggested compensatory adaptations to lower oxygen availability as previously described by Polícar et al., (2024). Overall, the observed biochemical, hematological and redox changes represent adaptive responses rather than stress-induced pathology, confirming the suitability and aptness of using higher stocking densities of pikeperch in IPRS.

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UTILIZATION OF BIOFUEL BYPRODUCTS: HEVEA SEED MEAL AND SAFFLOWER SEED CAKE AS ALTERNATIVE FEED INGREDIENTS FOR NILE TILAPIA (*Oreochromis niloticus*)

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Introduction

Between 2022-2024, 91% of total fish production in Sub-Saharan Africa originated from capture fisheries (Davids, 2026). The growth of aquaculture is projected to be 4.8% per annum, projected to the decline of capture fisheries to 87% by 2034. Therefore, there is still a significant potential in further development of aquaculture sector in the region.

Nile tilapia (*Oreochromis niloticus*) is a freshwater, fast-growing fish native to Africa. However, the demand for tilapia in African markets still mainly relies on imports. China leads in global tilapia production with a total export of 130,300 tonnes frozen whole tilapia within the first 9 months of 2025 (FAO, 2026). Côte d'Ivoire is the largest importer, while exports to Burkina Faso and Mali have also increased. In 2019, a smallholder tilapia farm in Mozambique received EUR 1.4 million investment from IDH Farmfit Fund (FAO et al., 2026), supporting the efforts to expand tilapia production in Africa.

Nevertheless, the aquaculture production in Africa still faces challenges in the production cost. Recent geopolitical tensions and energy market volatility have increased feed production costs affecting global supply chains. Therefore, there is a need to reduce the reliance on imported product by maximizing the utilization of locally-available ingredients. This can also lead to the reduction of aquaculture feed cost. Hevea trees (*Hevea brasiliensis*, from which natural rubber is obtained) and safflower (*Carthamus tinctorius*) are commonly cultivated in Africa, with Côte d'Ivoire ranking among top 5 largest natural rubber producer globally. Hevea and safflower seeds have been utilized for biofuel extraction in Africa. The by-product from hevea seeds processing (i.e. hevea seeds meal) contain 23.4 ± 1.1 g/100g protein, 2.88 ± 0.19 g/100g lipid, and 60.63 ± 1.2 g/100g carbohydrate. Meanwhile, by-product from safflower seeds processing (i.e. safflower seeds cake) contain 19.29 ± 0.93 g/100g protein, 19.1 ± 1.2 g/100g lipid, and 52.76 ± 1.54 g/100g carbohydrate. Despite their potential nutritional value, these by-products remain underutilized. Therefore, this study investigates the utilization of these byproducts extracted from hevea and safflower seeds, commonly utilized for biofuel production, as alternative feed ingredients for juvenile Nile tilapia.



Figure 1. Grinded hevea seeds meal (left) and safflower seeds meal (right).

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Materials and Methods

Rearing conditions

Nile tilapia larvae were obtained from Wageningen University, Netherlands, and reared at the Laboratory of Aquaculture, Department of Veterinary Medical Sciences, University of Bologna (Cesenatico, Italy). Larvae were fed commercial diet (Skretting, Perla Plus 2.0). When reached 20 ± 5 g (juvenile stage), 50 tilapia were randomly distributed to 21 tank connected to recirculated aquaculture system (RAS) (three replicates per dietary treatment) equipped with mechanical sand filtration, ultraviolet lights, and a biofiltration as described by Parma et al. (2023) and reared for 1.5 month. Water parameters were maintained at 24 ± 1.0 °C, 0.5 ± 0.5 g/L salinity and oxygen levels at 8.0 ± 1.0 mg/L using a liquid oxygen system.

Diet

To simulate the potential conditions of an aquafarm in remote areas of the African countries, where limited technological advancement is in place and cost of shipping of materials as well as the pelleted feed from accredited feed companies is prohibitive, diet was produced within the laboratory using an automatic pasta-making machine (La Monferrina P12). The resulting spaghetti-shaped pellet were blended and sieved to generate pellet of 1 to 2 mm. After production, pellets were dried in an oven.

Fish were fed twice daily using an automatic feeder six days per week, with 7 different isoproteic, isolipidic, and isoenergetic diets:

- Diet 1: control diet
- Diet 2: 10% inclusion of hevea seed meal
- Diet 3: 20% inclusion of hevea seed meal
- Diet 4: 30% inclusion of hevea seed meal
- Diet 5: 10% inclusion of safflower seed cake
- Diet 6: 20% inclusion of safflower seed cake
- Diet 7: 30% inclusion of safflower seed cake

Sampling

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. Additionally, gut microbiota and plasma biochemistry were also sampled and analyzed. Moreover, digestibility of hevea seed meal and safflower seed cake was analyzed with yttrium oxide in a 2-week period after the end of the feeding trial. 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 Discussions

Preliminary results suggest that the inclusion of hevea seed meal and safflower seed cake at 10 and 20% could do not significantly affect the growth performance of juvenile tilapia. The protein content of both hevea seed meal and safflower seed cake can adequately meet the dietary protein requirements, and the lipid content of safflower seeds meal can fulfil the dietary lipid needs. Negative impact on the growth performance was observed at 30%, which can be caused by the relatively high crude fibre content in the diets (Suprayudi et al., 2016).

Nevertheless, at 10 and 20% inclusion, hevea seed meal or safflower seed cake can lead to the replacement of 15-30% of soybean meal and 20-40% of wheat flour. Despite this, digestibility of these alternative ingredients is lower than that of conventional feed components. Notably, a diet containing 10% hevea seed meal and 10% safflower seed cake exhibits digestibility comparable to the control diet, whereas higher inclusion levels result in reduced overall diet digestibility. This decline in digestibility likely limits nutrient availability and absorption, thereby contributing to observed differences in growth performance.

The reduced digestibility and acceptance may be attributed to the presence of anti-nutritional factors, such as tannins and phytic acid, in both hevea seeds meal and safflower seeds cake. Additionally, processing methods, including dehulling and heat treatment, may further influence nutrient availability and palatability, thereby affecting feed utilization efficiency.

Conclusions

The preliminary findings support the utilization of hevea and safflower seeds from biofuel by-products as feed ingredients for Nile tilapia, reducing the dependence on imported soybean meal in Africa.

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INCLUSION EFFECTS OF JACK MACKEREL MEAL IN DIETS REPLACING 50% OF FISH MEAL WITH FREEZE-DRIED POLLOCK BY-PRODUCT MEAL ON THE GROWTH PERFORMANCE AND FEED AVAILABILITY OF OLIVE FLOUNDER *Paralichthys olivaceus*, AND ECONOMIC ANALYSIS

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Introduction & Methodology

The low-fish meal (FM) diets commonly cause reduced palatability and decreased feed consumption (FC), eventually impairing growth performance of fish (Hussain et al., 2024). Our previous study (Jeong et al., 2020) revealed that jack mackerel meal (JMM) exhibited the greatest feeding attractant response to olive flounder among 15 protein feed ingredients including various FMs and crustacean meals. Therefore, this study aims to evaluate the inclusion effects of JMM in diets replacing 50% of FM with freeze-dried pollock by-product meal (FPBM) on the growth performance and feed availability of olive flounder, and economic analysis. A total of 525 juvenile (averaging 6.3 g) fish were randomly distributed into 21 of 50-L flow-through tanks (25 fish/tank). Seven isoproteic (52.5%) and isolipidic (12.0%) diets were formulated. The control (Con) diet contained 60% FM. In the Con diet, 50% of FM was replaced with FPBM, and then increased levels (0%, 10%, 20%, 30%, 40%, and 50%) of JMM were included at the expense of FM, named as the FJ0, FJ10, FJ20, FJ30, FJ40, and FJ50 diets, respectively. All experimental diets were assigned to triplicate groups of fish. Fish were hand-fed twice a day for 50 days until visual satiation was reached.

Results & Discussion

At the end of the 50-day feeding trial, weight gain (WG) and specific growth rate (SGR) of fish fed the FJ40 and FJ50 diets were significantly ($P < 0.0001$ for both) higher than those of fish fed the FJ0, FJ10, and FJ20 diets, but not significantly ($P > 0.05$) different from those of fish fed the Con diet (Fig. 1). In orthogonal polynomial contrast, WG and SGR of fish revealed linear ($P < 0.0001$ for both) relationships with dietary inclusion levels of JMM in the low-FM diets replacing 50% of FM with FPBM. In regression analysis, linear models were determined to be the most suitable models between inclusion levels of JMM in diets replacing 50% of FM with FPBM versus WG ($Y = 1.174333X + 14.1832$, $P < 0.0001$, Adj. $R^2 = 0.8840$) and SGR ($Y = 0.000946X + 0.0239$, $P < 0.0001$, Adj. $R^2 = 0.7988$), respectively. FC of fish fed the FJ50 diet was significantly ($P < 0.0001$) higher than that of fish fed the FJ0 and FJ10 diets, but not significantly ($P > 0.05$) different from that of fish fed the Con diet (Fig. 2). In orthogonal polynomial contrast, FC of fish revealed linear ($P < 0.0001$) relationship with dietary inclusion levels of JMM in the low-FM diets. In orthogonal polynomial contrast, FC of fish revealed linear ($P < 0.0001$) relationship with dietary inclusion levels of JMM in the low-FM diets. In regression analysis, linear model was determined to be the most suitable model between inclusion levels of JMM in the low-FM diets versus FC ($Y = 0.791677X + 15.3158$, Adj. $R^2 = 0.7357$, $P < 0.0001$). Economic profit index (EPI) of fish fed the FJ40 and FJ50 diets was significantly ($P < 0.0001$) higher than that of fish fed the FJ0, FJ10, and FJ20 diets, but not significantly ($P > 0.05$) different from that of fish fed the Con diet. In orthogonal polynomial contrast, EPI of fish revealed linear ($P < 0.0001$) relationship with dietary inclusion levels of JMM in the low-FM diets. In regression analysis, linear model was determined to be the most suitable model between inclusion levels of JMM in low-FM diets versus EPI ($Y = 0.003857X + 0.7797$, Adj. $R^2 = 0.8999$, $P < 0.0001$). However, feed utilization (feed efficiency, protein efficiency ratio, and protein retention), biological indices, chemical composition, and amino acid profiles of fish were not significantly affected by dietary treatments. Incorporated 30–50% JMM as a feed enhancer in the low-FM diets replacing 50% of FM with FPBM achieved comparable WG, SGR, and FC to fish fed the Con diet. Furthermore, the FJ50 diet led to the highest EPI to farmers. In conclusion, the FJ50 diet seems to be the most recommended strategy for olive flounder farmers in terms of the highest EPI.

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Figure

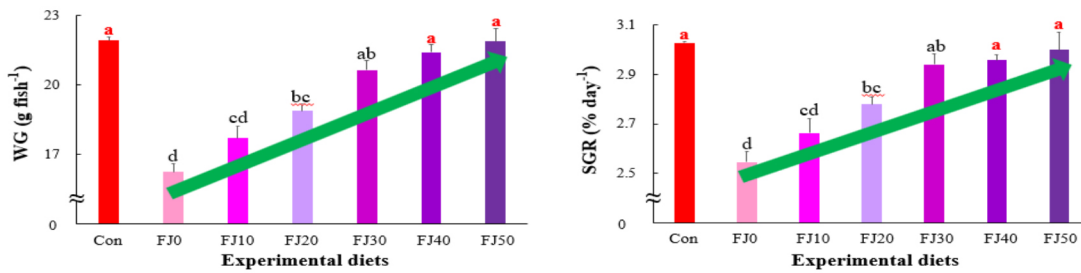


Fig. 1. Weight gain (WG, g fish⁻¹) (Polynomial orthogonal contrast; Linear = 0.0001, Quadratic = 0.1218, Cubic = 0.5018) (Regression analysis; $Y = 1.174333X + 14.1832$, Adj. $R^2 = 0.8840$, $P < 0.0001$) and specific growth rate (SGR, % day⁻¹) (Linear = 0.0001, Quadratic = 0.0936, Cubic = 0.5018) ($Y = 0.000946X + 0.0239$, Adj. $R^2 = 0.7988$, $P < 0.0001$) of olive flounder fed the experimental diets for 50 days (means of triplicate) ($P < 0.0001$ for both).

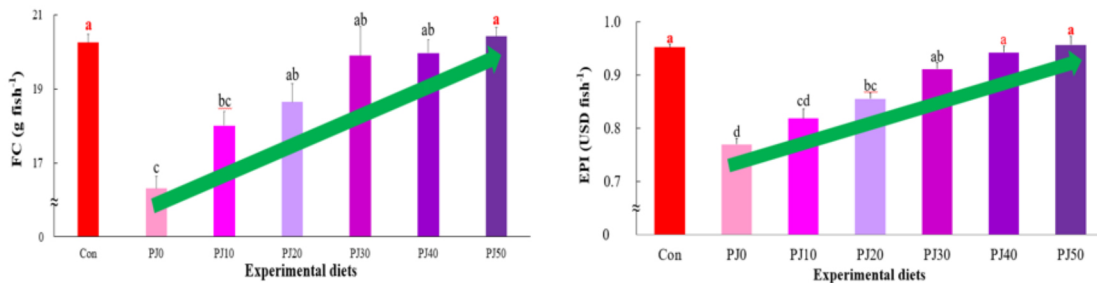


Fig. 2. Feed consumption (FC, g fish⁻¹) (Linear = 0.0001, Quadratic = 0.0663, Cubic = 0.7774) ($Y = 0.791677X + 15.3158$, Adj. $R^2 = 0.7357$, $P < 0.0001$) and economic profit index (EPI, USD fish⁻¹) (Linear = 0.0001, Quadratic = 0.1062, Cubic = 0.4563) ($Y = 0.003857X + 0.7797$, Adj. $R^2 = 0.8999$, $P < 0.0001$) of olive flounder fed the experimental diets for 50 days (means of triplicate) ($P < 0.0001$ for both).

Acknowledgment

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EVALUATION OF TRUE PROTEIN CONTENT AND AMINO ACID AVAILABILITY IN HIGH QUALITY FEED INGREDIENTS FOR RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

The protein content in feed ingredients is commonly estimated by multiplying total nitrogen by the nitrogen-to-protein conversion factor of 6.25, derived from the assumption that all proteins contain 16% nitrogen ($1/0.16 = 6.25$) and all measured nitrogen originates from protein. However, feed ingredients also contain non-protein nitrogen, and the protein nitrogen content varies considerably depending on the amino acid composition (Jones, 1941), introducing errors of up to 20% in protein estimations (Mariotti et al., 2008). Furthermore, knowing only the true protein content is insufficient for fish feed optimization. Determination of amino acid apparent digestibility coefficients (ADCs) is also required to assess how much of each amino acid is actually available to the fish. Therefore, this study aimed to evaluate the amino acid ADCs in rainbow trout (*Oncorhynchus mykiss*) fed on six high quality protein ingredients (fishmeal, blood meal, wheat gluten, maize gluten, soy protein concentrate, and pea protein concentrate), and obtain more accurate nitrogen-to-protein conversion factors from the complete amino acid profiles (Ka) and from the Kjeldahl-N content (Kp).

Materials and methods

Six isoenergetic diets (25 MJ kg⁻¹) were formulated: a fishmeal-based reference diet and five test diets where 25% of dietary protein was replaced by blood meal, wheat gluten, maize gluten, soy protein concentrate, or pea protein concentrate. Rainbow trout (~150 g) were distributed into 21 tanks (n=3) and fed the experimental diets for seven weeks. Fish were kept in a freshwater recirculating aquaculture facility (RAS) with optimal temperature, oxygen, and pH for rainbow trout. At the end of the trial, total biomass, individual weight, and fork length were recorded, and feces were collected by manual stripping. All twenty amino acids were quantified in feces, diets, and raw ingredients by UPLC to derive amino acid ADCs.

Results and discussion

ADCs of most amino acids differed significantly among ingredients. Wheat gluten showed the highest overall amino acid digestibility (98%), while soy protein concentrate showed the lowest (88%), suggesting differences in anti-nutritional factor content and protein structure among plant-based sources. Cysteine showed the greatest variation among ingredients, highlighting the importance of sulphur amino acid availability in precision feed formulation. Both Kp and Ka were considerably lower than the commonly applied conversion factor of 6.25, and while Kp did not differ significantly among ingredients, Ka did. However, although more precise, the use of Ka requires full amino acid profiling which is both costly and time-consuming. In conclusion, the study showed that the commonly applied nitrogen-to-protein conversion factor of 6.25 largely overestimates the protein content in aquafeed protein sources and that more accurate estimates can be obtained using ingredient-specific conversion factors, with Ka providing the most precise representation of true protein based on amino acid composition, while Kp offers a practical alternative for routine application when full amino acid profiling is not feasible.

Acknowledgment

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LONG TERM EFFECTS OF NANO-PVC PARTICLES ON BROWN TROUT, *Salmo trutta*

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Introduction

Microplastics (MPs) are ubiquitous environmental contaminants whose impacts are cumulative and long-lasting. In freshwater ecosystems, MPs originate from intentionally manufactured particles released during product use or industrial processes, the fragmentation and abrasion of mismanaged plastic waste, and tyre wear (D'Avignon et al. 2022). Following their release into the environment, MPs undergo various degradation processes, generating progressively smaller particles with an increased capacity to translocate across biological barriers and localize within cells. In fish, MPs have been associated with a wide range of adverse effects, including impaired intestinal function, oxidative stress, histopathological alterations, inflammation, and hematological disturbances (Islam et al. 2025).

The present study investigated the long-term effects of nano PVC spheres (diameters: 0.5 and 1 μm) in *Salmo trutta* six months after exposure. Specifically, the study determined whether PVC persisted in the fish, characterized their distribution among different organs, and assessed their effects on the expression of selected genes in the brain, kidney, spleen, and liver. In addition, blood cell composition was analysed to complement and validate the gene expression data. Further validation by analysis of selected protein biomarkers is currently in progress.

Material and Methods

Three-year-old *S. trutta* (body mass: 570 ± 190 g; total length: 38 ± 3 cm) were used. Fish were assigned to three groups of 60 individuals and stocked in separate concrete ponds supplied with 9°C groundwater at a flow rate of 2.0 L s^{-1} . All relevant water quality parameters were monitored and remained within the optimal range throughout the experiment. Spherical PVC particles (0.5 and 1 μm) were obtained from Abvigen (New Jersey, USA) and administered via feed. Incorporation of PVC into commercial trout feed was performed as described (Lahnsteiner et al. 2026). The administered PVC doses were as follows: low concentration, 250 mg PVC (1.5×10^{12} 0.5 μm particles and 0.2×10^{12} 1 μm particles); high concentration, 750 mg PVC (4.5×10^{12} 0.5 μm particles and 0.5×10^{12} 1 μm particles). Assuming complete feed consumption and homogeneous distribution within the fish, the expected tissue concentrations were $4 \mu\text{g g}^{-1}$ (50×10^6 0.5 μm spheres and 6×10^6 1 μm spheres g^{-1} tissue) for the low dose and $25 \mu\text{g g}^{-1}$ (150×10^6 0.5 μm spheres and 18×10^6 1 μm spheres g^{-1} tissue) for the high dose.

Following exposure, 15 fish from each treatment group were selected, tagged, and transferred to a single pond, where they were maintained for six months and fed a standard commercial trout diet. Subsequently, 10 fish per group were euthanized by electrical stunning, weighed, and measured. Brain, liver, spleen, and kidney tissues were excised and processed for microplastic detection using a published method (Lahnsteiner et al. 2026) and for gene expression analysis. Blood samples were collected for hematological analysis. Gene expression analysis followed standardized protocols using actin (*actb*) and hypoxanthine-guanine phosphoribosyltransferase (*hprt1*) as housekeeping genes. Data were analysed by one-way ANOVA with treatment as the independent variable.

Result

At the end of the experiment, fish body mass was 731 ± 116 g and total length was 48 ± 10 cm ($n = 30$), with no significant differences among treatment groups. No mortality was observed. MP particles corresponding to the administered 0.5 and 1 μm PVC spheres were detected in all examined organs of exposed fish but were absent from control fish. In contrast, other (foreign) MP particles were detected in tissues of all fish, including controls. The number of foreign MPs was significantly lower than that of the administered PVC particles. However, when converted to mass concentration, foreign MPs occurred at levels similar to those of the administered PVC because they were larger ($>1\text{--}10 \mu\text{m}$ in diameter). Both particle number and mass concentration of PVC MPs were highest in the kidney. The frequencies of 0.5 and 1 μm PVC particles were similar, although 0.5 μm particles showed a tendency to occur at higher abundance. In the spleen, expression of *epo* and *csfr1* was decreased, whereas *gata2* expression was increased at both PVC concentrations; *g-csfr* expression increased only at the low concentration. In the kidney, *fxyd6* and *aqp10b* expression increased following PVC exposure, whereas

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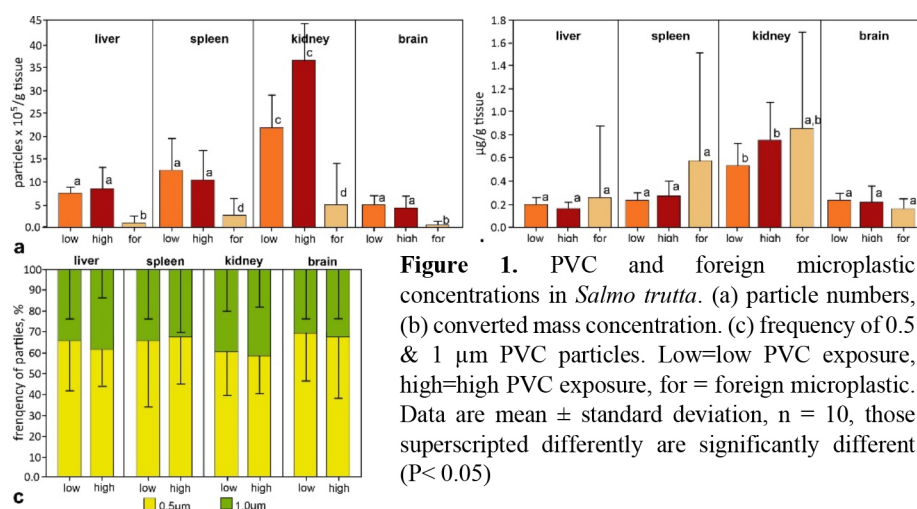


Figure 1. PVC and foreign microplastic concentrations in *Salmo trutta*. (a) particle numbers, (b) converted mass concentration. (c) frequency of 0.5 & 1 µm PVC particles. Low=low PVC exposure, high=high PVC exposure, for = foreign microplastic. Data are mean $\pm$ standard deviation, n = 10, those superscripted differently are significantly different ($P < 0.05$)

abcc1 expression decreased at the low concentration. In the liver, expression of *atp5l*, *fbxo32*, *ghra*, *igf1*, *il-6*, and *il-10* was reduced, whereas *cat* and *cyp2k1* expression increased in PVC-exposed fish. In the brain, expression of *egr1* and *fos* increased following PVC exposure, while *gabapap* expression decreased only at the high PVC concentration. Expression of kidney *xyd6*, liver *casp8* and *gsr*, and brain *calm2a* and *htr1a* did not differ significantly among treatments. Hematological analysis showed decreased hemoglobin concentration and reduced numbers of erythrocytes and granulocytes in both PVC-treated groups, whereas the number of blast cells increased. No significant changes were observed in other blood cell populations.

Discussion

(1) PVC microplastic particles persisted in the brain, kidney, liver, and spleen of exposed fish at high concentrations following a 6-month depuration period. (2) Six months after exposure, the expression of specific genes remained altered in all investigated organs, indicating long-lasting transcriptional effects of PVC microplastics. (3) Persistent effects were also evident at the hematological level, with reduced hemoglobin concentration and erythrocyte counts observed in exposed fish.

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ANALYSIS OF GROWTH PERFORMANCE AND TISSUE-SPECIFIC METABOLOMIC PROFILES IN WHITELEG SHRIMP (*Litopenaeus vannamei*) REARED IN RAS AND BIOFLOC SYSTEMS

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Introduction

As global shrimp aquaculture continues to expand, the industry faces increasing pressure to develop sustainable rearing systems that optimize feed efficiency and reduce environmental impact. While Recirculating Aquaculture Systems (RAS) are widely utilized for their high level of biosecurity and environmental control, Biofloc Technology (BFT) has emerged as a promising alternative, leveraging dense microbial communities to recycle nitrogenous waste into supplemental “*in situ*” nutrition (El-Sayed, 2021). Despite the known growth benefits of biofloc, the extent to which these systems influence the metabolomic status of whiteleg shrimp (*Litopenaeus vannamei*) remains poorly characterized. Understanding these shifts is crucial for improving husbandry protocols and final product quality; a useful tool to study the metabolome in relation to diverse dietary regimes is ¹H-NMR (Costantini et al., 2018). Using this approach, the goal of this study was to compare the growth performance and tissue-specific ¹H NMR metabolomic profiles of whiteleg shrimp reared in RAS vs. BIOFLOC systems under restricted feeding conditions, providing novel insights into the nutrient utilization and metabolic adaptation in intensive culture.

Materials and Method

Whiteleg shrimp (14.23 ± 0.51 g) were reared for 70 days in two distinct systems: a traditional Recirculating Aquaculture System (RAS) and a Biofloc-based system (BIOFLOC). Stocking density was maintained at 100 individuals m⁻³ across triplicate. While the RAS group was fed a standard commercial diet following the producer’s indication at a 2.8% daily feeding rate, the BIOFLOC group received a 20% restricted ration to account for the nutritional contribution of biofloc aggregates. Growth performance and feed efficiency were evaluated. At the conclusion of the trial, aqueous and lipid fractions of the muscle and hepatopancreas were extracted and analyzed using ¹H-NMR spectroscopy (Del Coco et al., 2018). Multivariate statistical analysis (PCA and OPLS-DA) was then applied to evaluate potential differences in metabolic profiles and identify specific biomarkers.

Results

Survival and growth indicators (FBW, WG, and SGR) did not differ significantly between treatments, with survival exceeding 92% in both groups. However, feed efficiency was significantly improved in the BIOFLOC group, which exhibited a lower apparent feed conversion rate (A-FCR: $p = 0.023$) despite a 20% reduction in administered feed. Multivariate statistical analysis of ¹H-NMR spectra revealed distinct metabolomic signatures between the two systems, (OPLS-DA models; $Q^2 > 0.85$). In the muscle extracts, BIOFLOC shrimp showed higher relative concentrations of specific amino acids (leucine, phenylalanine, arginine), 2-hydroxybutyrate, saturated FAs and phospholipids. Conversely, this group exhibited lower levels of glutamate, glycine, lactate, TMAO and unsaturated FAs compared RAS group. On the other hand, BIOFLOC hepatopancreas extracts showed a distinct shift characterized by increased in aspartate, 2-hydroxybutyrate, lactate, and unsaturated FAs, offset by lower levels of leucine, glycine, betaine, TMAO, choline, saturated FAs, and phospholipids (relative to RAS).

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Discussion

This study confirms that biofloc technology can maintain growth performance when the feeding rate is reduced by 20%. The results also show that the different rearing methods caused a shift in the shrimp's compositional profile. The high levels of essential amino acids (leucine, phenylalanine, and arginine) in the muscle, confirms that BIOFLOC aggregates can serve as a high-quality supplemental “in situ” nutritional source. On the contrary, the elevated levels of glutamate and TMAO in RAS shrimp may suggest a greater reliance on specific osmoregulatory pathways or different nitrogenous waste processing compared to the BIOFLOC group. Furthermore, the divergence in lipid composition, specifically the higher accumulation of phospholipids and saturated fatty acids in BIOFLOC-reared shrimp, indicates a shift in lipid metabolism and membrane structural components influenced by the microbial community. These results highlight that BIOFLOC-based systems enhance nutrient utilization and alter the nutritional “fingerprint” of the final product.

Acknowledgments

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FROM LANDING OBLIGATION TO FEED: LOCALIZED BOGUE AND HORSE MACKEREL MEALS IN MEDITERRANEAN MEAGRE (*Argyrosomus regius*) AQUACULTURE

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Introduction

To meet European circular economy goals and safeguard regional food security, aquaculture must transition away from imported marine ingredients toward localized, upcycled alternatives. Small pelagic species such as Bogue (*Boops boops*) and Horse Mackerel (*Trachurus trachurus*) represent major commercial fisheries in the Mediterranean that face intensive exploitation. When a selective fishing method it is not implemented, a significant fraction of these catches consists of unavoidable, undersized, over-quota, or damaged specimens that are strictly prohibited from entering the direct human food market (Pointin et al., 2019). However, under the mandates of the EU Landing Obligation (Article 15 of Regulation EU No 1380/2013), these specimens must be brought to port rather than returned to the sea (Uhlmann et al., 2018). Consequently, these 'regulatory discards' generate a high-quality marine protein waste stream that requires urgent valorization. Concurrently, Meagre (*Argyrosomus regius*) is a rapidly growing, high-potential Mediterranean species whose natural wild diet includes small pelagics, making the species an ideal candidate for trophic-matched regional diets. Therefore, the objective of this study was to investigate the biological feasibility of fully substituting standard imported commercial fishmeal with local, upcycled fishmeals derived from Bogue and Horse Mackerel discard fractions in the diet of juvenile Meagre.

Materials and Method

Juvenile meagre [42.7 ± 0.2 g mean body weight(BW)] were reared in a recirculating aquaculture system (RAS) at the AquaBioTech Group Innovia facility (Malta). Four isoproteic and isolipidic diets (48% CP, 15% CF) were evaluated: a standard control (CTRL) and three experimental diets featuring 100% imported fishmeal replacement using upcycled, locally sourced (FAO37, Malta) Bogue (BOG), Horse Mackerel (MAC), or a 50:50 mixture (MIX). Fish were fed twice daily to apparent satiation. Growth performance (BW, WG, SGR, FCR, SFR, DFI, SR), fillet proximate composition, and fatty acid profile were evaluated at trial termination.

Results

Over the 63-day trial, high survival rates and FCR were maintained across all treatments without significant differences. SGR was significantly higher in the MAC group compared to the CTRL group, with BOG and MIX exhibited intermediate values. Significant overall dietary effects on BW and WG, with numerical peaks observed in BOG (143.9 ± 2.0 g; 101.2 ± 1.8 g) and MAC (144.4 ± 2.0 g; 101.06 ± 1.7 g), though post-hoc pairwise comparisons were not significant. Daily feed intake and specific feeding rate showed no significant variation among groups. At trial termination, fillet proximate composition (dry matter, crude protein, crude lipid, and ash) showed no significant differences among dietary treatments. In contrast, fillet fatty acid profiles varied significantly; fish fed the MAC, BOG, and MIX diets exhibited lower levels of total saturated fatty acids and trans-fats, accompanied by higher proportions of monounsaturated, Omega-6, and Omega-9 fatty acids compared to the CTRL group.

Discussion

These findings demonstrate that local regulatory discards of Bogue and Horse Mackerel can be used in replacement of standard imported fishmeal in juvenile meagre diets without compromising survival, feed utilization efficiency, or nutrient deposition. The significant enhancement in growth rate observed in horse mackerel-fed fish, alongside positive numerical trends in weight gain for both single-ingredient meals, highlights the strong nutritional suitability and palatability of trophic-matched, regional pelagic raw materials. In addition, the shift in fillet fatty acid profiles toward lower saturated and trans-fatty acids, reflects the distinct lipid characteristics of these local species and suggests potential nutritional benefits for the final product. Overall, valorizing Mediterranean fisheries discards in accordance with the EU Landing Obligation could offer a viable strategy to advance circularity, reduce reliance on imported marine ingredients, and support production in the region.

(Continued on next page)

Acknowledgments

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AGRICULTURAL SIDE-STREAMS FERMENTED WITH *Ganoderma* spp. AS A NOVEL FUNCTIONAL INGREDIENT: ENHANCING FISH HEALTH AND GROWTH IN MEAGRE (*Argyrosomus regius*) AND EUROPEAN PERCH (*Perca fluviatilis*)

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Introduction

The aquaculture industry is a primary driver of global seafood production, traditionally depending on marine-derived resources to provide nutritionally balanced diets. However, the escalating cost of these raw materials and the environmental impact of their overexploitation present significant challenges to the sector's sustainability. Consequently, there is increasing interest in exploring the replacement of these materials with alternative feed ingredients. The aim of the present study is to address the sustainability challenges of the aquaculture sector by focusing on the circular valorization of agri-food side-streams as a substrate to produce a novel functional feed ingredient. Mycelium biomass derived from filamentous fungi is investigated as sustainable alternative to conventional feed ingredients. This study will specifically focuses on the application of mycoprotein derived from the mycelium of *Ganoderma* spp., a source characterized by a high crude protein concentration (> 50%), a balanced amino acid profile, and the absence of common plant-based anti-nutritive factors.

Materials and Methods

Solid state fermentation was used with low-value side-streams from the agrifood sector to produce filamentous fungi *Ganoderma* spp. The resulting fungal biomass will be used to replace fishmeal at increasing substitution levels of approximately 25%, 50%, and 75% in diets formulation for meagre (*Argyrosomus regius*, control diet with 20% fishmeal) and European perch (*Perca fluviatilis*, control diet with 23% fishmeal), resulting in mycoprotein inclusion levels ranging from 5.35% to 15.50% and 5.75% to 17.25%, respectively.

The research will be conducted at the AquaBioTech testing facility in Malta, using state-of-the-art recirculating aquaculture systems (RAS) to ensure strictly controlled environmental conditions. Long-term feeding trials will be executed to evaluate the impact of these novel diets on growth performance, feed efficiency, and product quality. Comprehensive health assessments will be performed, including histological analysis of digestive tissues and monitoring of physiological health parameters.

Preliminary and expected results

The solid state fermentation transformed the nutritional profile of the substrate, and promoted the production of bioactive secondary metabolites. An increase of the crude protein concentration, an improvement of the amino acid profile, and the elimination of anti-nutritive factors was obtained.

Specifically, the harvested *Ganoderma* spp. mycelium exhibited a high crude protein content of 53.3% (dry matter basis), a balanced amino acid profile rich in glutamic acid, aspartic acid, leucine, and lysine, alongside a relatively low crude fat content (3.9% DM) and a crude fiber content of 8.5% DM (NDF 19.8% DM), with a high insoluble structural fraction (ADL 10.1% DM) attributed to fungal cell wall chitin and glucan complexes. The experimental feeds are currently under preparation and the trials are expected to start in autumn 2026.

Beyond basic nutrition, bioactive secondary metabolites contained in the mycellium, including β -glucans, triterpenes, and phenols, are expected to provide potent health-enhancing properties and improve the immune system capacity.

Acknowledgment

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PRECISION FERMENTATION DERIVED FUNCTIONAL NUTRITION IMPROVES STRESS RESILIENCE AND RECOVERY IN ATLANTIC SALMON (*Salmo salar*)

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Introduction

Stress associated with smoltification, handling, grading, transport and sea lice management can reduce feed intake, suppress growth and increase performance variability in Atlantic salmon (*Salmo salar*). Nutritional strategies that support fish performance during and following these events are therefore of increasing interest. ME-PRO is a precision-fermented soybean protein ingredient characterized by low acid-binding capacity, enhanced nutrient digestibility and the presence of fermentation-derived bioactive compounds including arabinoxylan oligosaccharides (AXOS), beta-glucans, organic acids and isoflavones. The objective of this work was to evaluate the effects of ME-PRO on nutrient utilization, growth performance and stress resilience in Atlantic salmon.

Materials and Methods

Atlantic salmon were reared in a recirculating aquaculture system and fed diets containing 10% or 15% ME-PRO, soy protein concentrate (SPC), a fishmeal-based control diet or a commercial reference diet. Growth performance and whole-diet digestibility were evaluated over a 15-week period. In a separate study, fish were subjected to repeated handling and crowding events three times weekly combined with a salinity transition from freshwater to 32 ppt over a two-week challenge period. Specific growth rate (SGR), feed intake, survival and post-stress recovery were evaluated.

Results and Discussion

ME-PRO improved phosphorus digestibility (50.9–53.1% versus 38.8–46.8%) and zinc digestibility (14.0–19.4% versus 5.3–12.5%) compared with SPC based diets while maintaining growth performance despite reduced fishmeal inclusion. During the stress challenge, salmon receiving 10% ME-PRO maintained positive growth (SGR 0.15% day⁻¹), whereas growth ceased in fish receiving the fishmeal based control diet. Feed intake declined in all groups during stress exposure but remained higher in fish receiving ME-PRO and recovered more rapidly following the challenge. Survival was numerically higher in the ME-PRO group (90% versus 85%). Additional observations from complementary studies indicated lower cortisol responses during stress events and a faster return toward baseline growth rates following stress exposure. Collectively, these findings suggest that fermentation-derived functional nutrition may support both resilience during stress and recovery following stress events.

Conclusion

Atlantic salmon receiving diets containing ME-PRO maintained growth during repeated environmental and husbandry stressors. These same salmon also demonstrated improved recovery characteristics following challenge.

The results of the aforementioned study indicate that functional ingredients produced through precision fermentation provide benefits beyond nutrient supply alone. These benefits directly support biological robustness, nutrient utilization, and production efficiency under commercial farming conditions.

Acknowledgement

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eDNA AND MACHINE LEARNING FOR ENVIRONMENTAL MONITORING – EXAMPLES FROM OFFSHORE TUNA FATTENING IN THE BAY OF BISCAY, SALMON FARMING IN NORWAY AND MORE

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Routine biomonitoring is key to understanding how aquaculture affects the marine environment and the ecosystems around it. Accurate identification of marine species is essential for understanding biodiversity and ecosystem health, and benthic macroinvertebrates (macrobenthos) are widely used as indicators of environmental change in monitoring programs. However, their identification through morphology is labour-intensive and requires specialized taxonomic expertise. A promising alternative to this is environmental DNA (eDNA) sequencing, which detects traces of genetic material left behind by organisms in the environment, or, when sufficiently small, the organisms themselves. This approach – especially when focused on microorganisms – has already shown strong potential in aquaculture and other fields.

Some recent studies have moved beyond large bottom-dwelling animals (macrobenthos) and started looking at much smaller organisms, such as microbes and meiofauna. These groups are particularly attractive as bioindicators because they respond quickly to environmental changes, especially factors like redox potential and nutrient availability. Their small size and more even distribution in sediments also make them easier to study using eDNA based methods. However, this presents a challenge; unlike larger organisms, we still lack well-established biotic indices and indicator species for microbes and meiofauna (with some notable exceptions such as microgAMBI and nema-gAMBI). This is where data-driven approaches come in. Instead of relying on predefined indicator species, these methods use patterns in the data to build new tools for environmental assessment.

One such approach is Random Forest Regression (RFR), a machine learning method that has gained considerable attention recently for its ability to predict ecosystem quality. It has already been applied in salmon aquaculture, and we have recently tested it in a novel offshore tuna farming installation in the Bay of Biscay. Here, I summarize the results of these studies, with a particular focus on using physicochemical pressure indices as a less biased alternative to traditional morphology-based indices when defining “ground truth”.

THE COSTS OF AN INVASION: HOW THE BLUE CRAB IMPAIRED ECOSYSTEM SERVICES IN THE MOST PRODUCTIVE LAGOON OF NORTHWESTERN ADRIATIC

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The introduction of non-native species can disrupt ecological processes and impair ecosystem services, particularly in intensively exploited systems such as coastal lagoons. The Atlantic blue crab (*Callinectes sapidus*) has recently expanded rapidly in the northern Adriatic, raising ecological and economic concerns. This study documents and evaluates its impact on provisioning services in the Sacca di Goro lagoon (Po Delta, Northern Italy), the most productive site for Manila clam in Europe. Net revenues from the emerging blue crab supply chain were also assessed by accounting for sales and disposal costs. Crab abundance showed no association with environmental variables. By contrast, a statistically significant negative relationship was detected with Manila clam production, which declined by 71.8 %, corresponding to annual losses of about €65 million. Small-scale fisheries also recorded reduced catches of native and commercially valuable species, with estimated losses of €0.18 million yr⁻¹. Revenues from the blue crab supply chain were positive in 2023 (+€0.41 million yr⁻¹) but turned negative in 2024 (- €0.71 million yr⁻¹) due to disposal costs and falling market prices. Thus, to date, not only are the revenues from blue crab sales negligible, but they are also outweighed by the high costs of disposal. This case shows how invasive species can simultaneously erode biodiversity and ecosystem services while failing to provide viable alternatives. It provides quantitative evidence of aquatic invasion costs and highlights the importance of integrating such assessments into ecosystem-based management and policy.

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ECO FEED FORMULATION FOR RAINBOW TROUT BREEDERS (PEPITO PROJECT)

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Introduction

Even though the rainbow trout sector represents 96% of the farmed fish production in France (roughly 39,000 tons in 2022 ; Pêche et Aquaculture, 2022), it is criticized for its environmental impacts like the other sectors of animal production (Wilfart *et al.*, 2023). Life Cycle Assessment (LCA) enables to evaluate some of the environmental impacts of aquaculture such as its impact on climate change and eutrophication. Moreover, LCA uses new indicators to study for example the impacts of aquaculture feeds on halieutic resources. In fact, fish feeds have been identified as the element of the life cycle mostly contributing to the impacts of aquaculture systems (25 to 95% of the studied impacts ; Wilfart *et al.*, 2019). One of the ways to reduce these environmental impacts is by using eco-formulated feeds (Wilfart *et al.*, 2023). If many studies have been conducted on the growing stages, a lot less have been dedicated to the production of fertilised rainbow trout eggs even though most of the fish farmers buy their fertilised eggs to specialised fish farms (Pêche et Aquaculture, 2022). Thus, it is essential for this sector to develop and promote production methods that reduce its environmental impacts. The main goal of the PEPITO project, a FEAMPA project financed by the European Union, the French government and Brittany, is to use multi-objective formulation (MOF) to design eco-feeds for rainbow trout breeders. MOF uses simultaneously the nutritional needs of rainbow trout breeders, environmental durability criteria and economical data.

Material and Methods

For this project, the MOF algorithm used is adapted from the one developed by Garcia-Launay *et al.* (2018) and modified by Wilfart *et al.* (2023). The nutritional, incorporation and economical constraints were given by Le Gouessant Aquaculture and modified after several technical meetings to increase the range of solution for the algorithm. The list of raw material was also given by Le Gouessant Aquaculture and supplemented with data collected from other feed manufacturers or with data from literature. The environmental constraints are calculated with the LCA impacts of the raw materials.

Two eco-feeds issued from the algorithm were selected for the trial and their performances will be compared with two commercial feeds. The impacts studied are the ones of EF 3.1 supplemented with Net Primary Production Use (NPPU) and Phosphorus Consumption (PC).

Results

The first results show a difficulty to obtain eco-feeds for rainbow trout breeders having a global environmental impact lower than the commercial feed, contrary to the results of the study conducted on the growing stage. The eco-feeds have a higher contribution on most of the studied impacts, except for NPPU, which is reduced by around 70%. Wheat gluten and soybean concentrate, which respectively represent around 5 and 15% of the feeds, are important contributors to the studied impacts, representing 10 to 40% of the studied impacts. Fish meal and fish oil represents more than 97% of the impact on NPPU.

Discussion

This study shows that applying important nutritional constraints to formulate feeds for breeders lead to a difficulty to produce feeds with lower environmental impacts than the commercial feeds. This is due to the partial substitution of fish meal and fish oil, highly incorporated in breeders commercial feeds, with plant-based ingredients. In fact, plant-based ingredients have higher environmental impacts than fish meal and fish oil for most of the impacts studied, especially land use, ecotoxicology and climate change. However, plant-based ingredients are beneficial for the preservation of halieutic resources, observed through the NPPU indicator. The next steps of the project are the trials, which aim to check the zootechnical performances of the eco-feeds, and the conception of an eco-feed for rainbow trout larvae.

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GENETIC AND ENVIRONMENT INTERACTION FOR SURVIVAL ASSOCIATED WITH *VIBRIO AESTUARIANUS* IN ADULT CUPPED OYSTERS IN FRANCE

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Introduction

Cupped oyster (*Crassostrea [Magallana] gigas* Thunberg, 1793) production is regularly threatened by episodes of mass mortality from spat to adult, representing a major economic issue for the oyster farming industry (Jourdan et al., 2026). Among the pathogens involved in mortality events in adult oysters, the bacterium *Vibrio aestuarianus* has been identified in various environments (Degremont et al., 2019). Consequently, the onset and intensity of mortality events often result from interactions between the bacterium, the environment, and the genetic and farming-related factors of the populations (Travers et al., 2014). The genetic resistance to *V. aestuarianus* was estimated as moderately heritable ($h^2=0.2$) in a genetic design based on separated families and a controlled challenge in laboratory (Azéma et al., 2017). The estimation of heritability in commercial conditions according to different rearing conditions and/or environments is not documented. The aim of this study was to estimate the selection potential for adult survival in the cupped oyster in different production environments to optimize breeding programs.

Material and Methods

300 families were produced by artificial fertilization and the size of maternal families was balanced before pooling all larvae into one cohort. At 18 months, 2 060 progeny were electronically tagged and biopsied (mantle) for later genotyping (as their parents). Three batches were created at 24 months and reared in subtidal environments: one in Normandy (Asnelles; 687 oysters) and one on the Atlantic coast in Vendée (Bay of Bourgneuf; 579 oysters). The third batch was reared suspended in the Mediterranean Sea (Thau Lagoon; 794 oysters). Parents and progeny were genotyped on the 40k Axiom Oyster Genotyping Array. Parentage assignment was performed using the R package APIS (Griot et al., 2020) to reconstruct the pedigree. Genetic parameters for survival were estimated within and between sites using a single-step threshold model with the THRGIBBSF90 software (Tsuruta & Misztal, 2006).

Results

In the Thau Lagoon, mortality was intermediate (33%), as in Normandy (24%), and was limited in Vendée (12%). Heritability estimates for survival in the three different sites were about 0.26-0.27, while overall survival, regardless of sites, was estimated at 0.21 ± 0.04 . Genetic correlations for survival were low to moderate (0.09 ± 0.2 to 0.58 ± 0.1) across sites (Table 1). The highest correlation was observed between Normandy and Thau, and the lowest was observed between Thau and Vendée.

Table 1 : Heritability (diagonal) and genetic correlation (above diagonal) for survival between site and ages estimated in GBLUP

	30 months- Normandy	30 months - Thau	30 months - Vendée
30 months -Normandy	0.26 [0.06]	0.58 [0.13]	0.23 [0.23]
30 months -Thau		0.27 [0.06]	0.09 [0.20]
30 months -Vendée			0.27 [0.07]

(Continued on next page)

Discussion

This study provides the first estimates of genetic parameters and GxE interactions of adult survival of cupped oysters across different commercial rearing conditions. The moderate heritabilities estimated for survival within and between sites confirm that survival, and probably also resistance to *V. aestuarianus*, in adult oysters can be genetically improved by genetic selection.

The intermediate to low genetic correlations between across environments suggest differences in the selection potential in each condition. The correlation between Normandy and Thau indicates that genetic gain can be expected across these sites, while the correlation between Thau and Vendée highlights the need to consider specific site effects. However, estimates involving the Vendée site should be interpreted with caution due to the low mortality and high standard errors (up to 0.26), suggesting limited accuracy.

Additional genetic analyses considering inter-annual variation within and between sites and across generations are necessary to better evaluate the importance of these and their impact on the expected genetic gain. Genetic correlation across rearing conditions should be taken into account to organize selection by site and optimize oyster breeding programs within the limit of economic barrier.

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HIGHER DIETARY LEVELS OF EPA & DHA IMPROVE SHRIMP (*Penaeus vannamei*) HEALTH, PERFORMANCE, NUTRITIONAL QUALITY AND REDUCE ENVIRONMENTAL IMPACT

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Introduction

It is increasingly recognised that long chain Omega-3 fatty acids EPA & DHA are essential for optimal shrimp health, performance and final product quality. However, the progressive reduction of marine ingredients in aquafeeds has decreased the natural supply of these fatty acids. This research focuses on understanding how restoring the levels of dietary EPA & DHA can support shrimp robustness, production efficiencies, nutritional value and environmental impact, enabling the sustainable growth of the industry.

Method

Two different shrimp trials were conducted to define the optimum EPA & DHA dietary levels to maximize growth, robustness, and nutritional quality. In the first trial (IMAQUA, Belgium) a control diet (0.4 % EPA & DHA) was compared to two diets (1% HB and 1% HA) formulated with a blend of fish oil and algae oil, or algae oil alone, respectively. At the end of the experimental feeding (56 days) shrimp growth, resistance to *Vibrio paraahaemolyticus* (which causes Early Mortality Syndrome (EMA) in shrimp) immune parameters, salinity stress resistance, and muscle fatty acid profiles were assessed. A second trial (SPAROS, Portugal) evaluated the benefits of graded dietary EPA & DHA levels (0.4 - 1.7%) on growth performance, nutrient utilization, stress resilience and tissue fatty acid composition using algae oil in fully fish oil-free diets. The environmental impact of increasing dietary EPA & DHA was modelled using Sustell™, a Life Cycle Assessment (LCA) platform for calculating the environmental footprint of both the feed and the shrimp.

Results

The studies confirmed that dietary levels of 1% EPA & DHA were consistently associated with improvements across key production metrics: shrimp receiving 1% EPA & DHA levels demonstrated improved survival and robustness (up to 23%), reflecting an enhanced health status under biological and environmental challenge conditions. Growth performance (up to 22%) and feed efficiency (up to 10%) were also positively affected, contributing to improved production efficiencies. The improved survival was modelled in Sustell™, resulting in a reduced carbon footprint of up to 15%. Importantly, increasing dietary inclusion from 0.4% to 1% EPA & DHA resulted in a doubling of the deposition in the edible muscle, enhancing the nutritional quality and value of the final product for human consumption.

Conclusions

These findings highlight the need to optimise EPA & DHA nutrition in modern shrimp farming.

- Improvements in health and survival directly support animal welfare, improving biological performance, enhancing productivity and increasing economic results.
- Better zootechnical performance contribute to a lower environmental footprint through more efficient use of resources.
- Increasing EPA & DHA levels in shrimp diets increased the nutritional value of farmed shrimp as a source of longchain Omega-3s and may create new market opportunities for producers.

Renewable, scalable sources of EPA & DHA offer a pathway to secure these benefits without relying on limited marine ingredients, while supporting the sustainable growth of the shrimp aquaculture sector.

THE REGENERATIVE BLUE ECONOMY FRAMEWORK: AN OPPORTUNITY TO ACCELERATE THE TRANSITION OF AQUACULTURE SYSTEMS TOWARDS STRONGER SUSTAINABILITY, OCEAN-POSITIVE IMPACTS, AND A PARADIGM SHIFT

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Introduction

In the context of increasing global demand for seafood products, aquaculture—now representing more than 50% of global seafood production—is widely seen as a viable economic development solution compared to fisheries, particularly in coastal areas. However, this is not the case in Europe, where access to new production sites has become increasingly constrained for aquaculture producers. The aquaculture sector also faces growing pressure to improve its environmental and social sustainability, as it is often perceived as contributing to biodiversity loss and social tensions within local communities, while competing with other marine activities and uses.

The *Kunming–Montreal Global Biodiversity Framework* highlights the need for innovative approaches in coastal areas that simultaneously support marine ecosystem conservation, food security, and social inclusion, particularly in relation to the 30×30 target. Similarly, the 2026 report of the Intergovernmental Platform on Biodiversity and Ecosystem Services (IPBES) calls for transformative changes across all economic sectors. This evolving global context presents significant challenges for aquaculture, but also creates an opportunity for a paradigm shift in its development.

Materials and methods

This study examines the Regenerative Blue Economy (RBE) framework, as introduced by Le Gouvello and Simard (2024) and recently endorsed through an International Union for Conservation of Nature (IUCN) resolution adopted in October 2025 (IUCN, 2025), as a comprehensive and operational approach to guide and accelerate sustainability transitions in marine aquaculture social–ecological systems. Positioned at the science–policy interface, it explores how RBE principles can support a transition towards ocean-positive aquaculture practices that not only minimise environmental impacts but also restore ecosystem functions and promote equitable access to marine resources and benefits for coastal communities.

RBE principles are analysed in relation to complementary aquaculture approaches aimed at enhancing food system sustainability, including restorative or regenerative aquaculture (Alleway et al. 2023; Mizuta et al. 2023), ecological aquaculture (Costa-Pierce, 2021), marine permaculture (Spillias et al. 2024), conservation aquaculture, aquaculture as a Nature-based Solution (Le Gouvello et al. 2023), and aquaculture within Marine Protected Areas or associated with Other Effective Area-Based Conservation Measures (OECMs) (Le Gouvello et al. 2017). Using RBE principles as an analytical lens, strengths and limitations are identified across case studies of aquaculture systems: low-trophic aquaculture (shellfish and algae) in Europe, integrated multi-trophic aquaculture in China, and community-based clam restocking in a locally managed marine protected area in Senegal. Across these cases, key enabling conditions and success factors are identified to inform both policy and practice.

Results and discussion

Results highlight the importance of developing integrated regional and national RBE strategies that connect aquaculture with other marine sectors, including fisheries, tourism, marine renewable energy, blue infrastructure, and marine conservation initiatives, within a broader transition towards ocean regeneration. Approaches such as Marine Spatial Planning and Integrated Coastal Zone Management, combined with policy instruments such as the European Union (EU) Restoration Law and the EU Ocean Pact, are identified as critical levers. Innovative financing mechanisms, including nature credits and partnership-based approaches, are also discussed.

The paper finally proposes a preliminary research and policy agenda, including new performance indicators to assess the contribution of aquaculture systems to regenerative and equitable marine development. It further discusses the challenges of aligning more intensive aquaculture models with regenerative principles and identifies priorities for future interdisciplinary research. The paper advocates for alignment between the future European 2040 aquaculture strategy and EU Ocean Act and the internationally recognised IUCN resolution on the Regenerative Blue Economy.

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GLOBAL RESEARCH TRENDS IN FISH POLYCULTURE AND PATHWAYS FOR DESIGNING NEW SYSTEMS

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Introduction

Fish polyculture is defined as the concurrent rearing of two or more species within the same aquaculture system at the same time. It is one of the oldest and most adaptable approaches for increasing aquatic food production. Despite its long history and recognized ecological and economic benefits, a comprehensive understanding of global research trends in fish polyculture has been lacking. Through a systematic review of scientific literature published between 1968 and 2023, we analyzed 889 peer-reviewed studies and produced the first global overview of fish polyculture research. This presentation synthesizes these trends and discusses how decision-support tools can facilitate the development of future polyculture systems.

Global Research Trends

The review revealed a strong geographic imbalance in research effort, with most studies conducted in Asia, while Africa, Europe, and the Americas remain comparatively underrepresented. Freshwater pond systems overwhelmingly dominated the literature, reflecting the historical importance of carp- and tilapia-based farming systems. Research objectives were primarily centered on growth performance, biomass yield, and feed utilization, whereas fewer studies investigated ecological processes, fish welfare, economic resilience, or social acceptability.

Temporal analysis nevertheless indicates an important transition in recent years. Increasing numbers of studies now address sustainability-oriented systems, including integrated multi-trophic aquaculture, biofloc-based production, and rice–fish farming. Emerging themes also link species diversity with nutrient recycling, improved trophic efficiency, and reduced environmental impacts. These developments suggest that polyculture is evolving from a productivity-driven practice toward a multifunctional strategy aligned with circular economy principles.

Future Development of New Polycultures

Although interest in polyculture is increasing, designing new fish species combinations remains complex because species may interact positively through niche complementarity or negatively through competition, stress, or predation. To overcome these constraints, structured decision-support approaches are needed. We developed an integrative three-step workflow consisting of: (1) selection of promising fish species combinations according to stakeholder objectives, (2) evaluation of environmental compatibility, welfare, and interaction risks, and (3) pilot-scale validation under farming conditions. We operationalized this workflow through several decision-support tools using *in silico* approaches. AquaDesign is an *in silico* tool that identifies abiotic farming conditions suitable for individual species and species combinations using ecological niche modeling. It can rapidly screen candidate species for new environments or emerging farming sectors. Predish is a complementary tool that estimates predation risk between fish species based on body-size relationships, helping to avoid combinations with high predation risk before experimental trials. Competifish is a third tool that uses functional fish trait information from public databases to estimate the risk of interspecific competition for trophic and spatiotemporal resources.

Conclusion

The global literature demonstrates that fish polyculture research has expanded substantially but remains geographically uneven and strongly focused on conventional freshwater production metrics. Future progress should prioritize underrepresented regions, broader production systems, and indicators linked to ecosystem functioning, welfare, and socioeconomic sustainability. Decision-support tools such as AquaDesign, Predish, and Competifish offer practical means to accelerate innovation while reducing costly empirical testing. Combining evidence from past research with predictive tools can help develop the next generation of resilient and sustainable fish polyculture systems.

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EARLY DOMESTICATION ALTERS PHYSIOLOGICAL STRESS RESPONSES AND PATHOGEN RESISTANCE WITHOUT IMPAIRING THERMAL TOLERANCE OR BEHAVIOR IN ZEBRAFISH *Danio rerio*: IMPLICATIONS FOR EX-SITU CONSERVATION

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Captive breeding is widely used to support ex-situ conservation and population reinforcement programs, yet adaptation to captive conditions can occur rapidly and alter traits essential for survival after release. Understanding how stress-coping phenotypes change during the early stages of domestication is therefore critical for assessing the long-term effectiveness of conservation breeding.

Wild zebrafish (*Danio rerio*) were collected from their natural habitat (F0) and subsequently bred under captive conditions for four generations (F1–F4). Across generations, we quantified multiple traits associated with resilience to environmental challenges relevant to reintroduction, including thermal tolerance (critical thermal maximum, CT_{max}), resistance to viral infection, behavioral responses (exploration, boldness, aggression and stress-related behaviors), physiological stress responses measured by waterborne cortisol release, and Fulton's condition factor (FCF). Data were analyzed using generalized linear models (binomial GLM for viral mortality), generalized linear mixed models (beta and negative binomial GLMMs for behavioral traits), and linear mixed models (LMMs for cortisol and FCF), followed by Tukey's post hoc tests with Benjamini-Hochberg correction where appropriate.

Captive breeding induced rapid but trait-specific phenotypic changes. Resistance to viral infection differed significantly among generations, with F4 exhibiting significantly higher mortality following viral challenge than F1–F3 ($\chi^2 = 21.10$, $p < 0.001$). Physiological stress responses also changed progressively, as both absolute and body mass-normalized waterborne cortisol release increased across generations and reached their highest values in F4 ($p < 0.001$). FCF increased significantly with domestication, whereas CT_{max} remained largely conserved across generations. Behavioral traits showed substantial individual variation, but no consistent directional changes remained significant after correction for multiple testing.

These findings demonstrate that substantial phenotypic changes can arise within only a few generations of captive breeding, but that domestication affects biological functions differently. While thermal tolerance and behavior remained relatively stable, physiological stress responses and pathogen resistance changed markedly during early domestication. Our results emphasize the importance of monitoring multiple fitness-related traits in conservation breeding programs and suggest that minimizing the duration of captive breeding before release may help preserve the adaptive capacity of fish reintroduced into natural environments.

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A PROJECT TO STUDY THE EFFECTS OF AQUACULTURE ENVIRONMENTS ON EARLY DOMESTICATION TRAJECTORIES IN ZEBRAFISH *Danio rerio*

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Aquaculture plays a pivotal role in global food security by providing an increasing share of aquatic animal protein (FAO, 2024). However, production remains concentrated on a limited number of intensively farmed species and standardized production systems, reducing the resilience and sustainability of the sector (Cai et al., 2023; Thomas et al., 2021). Diversifying both farmed species and farming environments is therefore considered a major challenge for the future of aquaculture. Domestication is a multigenerational process driven by interactions between genetic variation and the rearing environment, resulting in progressive changes in growth, behaviour, reproduction, stress responsiveness and other fitness-related traits (Milla et al., 2021). Despite the recognized importance of environmental effects, most domestication studies have been conducted under a single rearing condition, leaving the influence of alternative aquaculture environments largely unexplored (Lorenzen et al., 2012). This project aims to determine whether contrasting aquaculture environments generate distinct early domestication trajectories by following four generations of a model species zebrafish (*Danio rerio*) originating from a common wild population and reared under three aquaculture-inspired production systems.

Wild zebrafish collected in Bangladesh (F0) will be used to establish experimental populations maintained for four successive generations (F1–F4). From 28 to 140 days post-fertilization, fish will be reared under three contrasting environments: (i) standard monoculture without structural enrichment (NE), (ii) structurally enriched monoculture with artificial plants (PE), and (iii) polyculture with guppies (*Poecilia wingei*) (SEG). These treatments were designed to modify habitat complexity and social interactions while maintaining comparable water quality and feeding conditions. Each generation will be produced through controlled interfamilial crosses originating from ten unrelated founder pairs, with related matings systematically avoided to minimize inbreeding. A comprehensive phenotyping approach will assess survival, growth, morphology, behaviour, stress responsiveness, immune function, reproductive performance and brain morphology across generations and environments. These phenotypic data will be integrated with gonadal transcriptomics, brain genomic and epigenomic analyses, and gut microbiome profiling to identify the molecular mechanisms underlying environmentally driven domestication trajectories.

Acknowledgment

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EFFECTS OF PLANT SPECIES (LETTUCE, *Lactuca sativa* ; BASIL, *Ocimum basilicum*) ON THE GROWTH, WELFARE, AND PHYSIOLOGICAL STATUS OF THE EURASIAN PERCH (*Perca fluviatilis*) AND PLANTS, IN AQUAPONICS

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Introduction

Aquaponics is a soilless food production system that combines hydroponics and aquaculture to co-cultivate fish and plants in a closed-loop water system (Lama et al., 2025). It works by using fish waste, which is converted by microbial communities in the biofilter into nutrients that plants can absorb.

One aspect of this system is the potential for chemical communication (allelopathy) among plants, fish, and microorganisms (Senff et al., 2022). Plant roots release rhizodeposits composed mainly of carbon and nitrogen-rich organic compounds that stimulate microbial growth, as well as secondary metabolites that may influence organismal growth and animal welfare. The effects of these biomolecules depend on their concentration, their interactions, and the composition of the rhizodeposits, which is itself influenced by the plant species, developmental stage, and environmental conditions (Badri and Vivanco, 2009; McLaughlin et al., 2023).

The choice of plant–fish associations, as well as the use of monocultures or mixed plant cultures, is therefore critical for the proper functioning of the system. To date, no information is available on the diversity, concentrations, or effects of these biomolecules in aquaponic systems operating as coupled recirculating aquaculture systems (RAS).

Materials and Methods

Two crop production cycles, each lasting 6 weeks, were conducted consecutively in the 12 independent experimental aquaponic units in the greenhouse (Experimental Platform in Aquaculture, EPA) at the IUT Nancy-Brabois (54600 Villers-lès-Nancy, France). Each unit has a capacity of 365L and contains i) a 100L resin hydroponic compartment using the DWC (deep water culture) method on floating rafts, ii) a 210L glass aquaculture compartment, and iii) a 55L glass biofiltration tank containing 6 pieces of expanded polyurethane foam, serving simultaneously as a physical filter and a biofilter. **Monocultures** (12 plants per pilot) of **lettuce** and **basil**, as well as a **lettuce-basil polyculture** (6/6 per pilot), were grown in 4 replicates each, with 600g of perch (*Perca fluviatilis*) fed progressively up to 1.5% of their biomass. Water parameters were monitored regularly i) to check the nitrogen cycle (nitrite and ammonium concentrations), water temperature, and oxygen levels required for the well-being of the perch; and ii) to quantify the levels of macro- and micronutrients necessary for plant growth.

Mortality was checked every day and growth measurements of the perch (weight, total length, Fulton index) were taken every 3 weeks (5 fish per tank). At the end, specific growth rate was calculated and fin integrity (image analysis) analysed. The plants were monitored weekly for their morphology (height, number of leaves) as well as their physiological parameters (chlorophyll, anthocyanin, and flavonoid pigmentation; Nitrogen Flavonol Index; photosynthetic activity and stomatal conductance). At the end of the experiment, above-ground and root biomass, as well as root architecture, were measured.

Results

The study revealed mortality rates ranging from 5% to 32% per pilot. However, these values were not dependent on the treatment group ($p = 0.49$). Significant effects were recorded on Eurasian perch. Fish length was significantly greater in the polyculture treatment than in the lettuce monoculture ($p = 0.01$) and the Fulton index was highest in the lettuce monoculture treatment ($p = 0.002$ compared to the basil monoculture and $p = 0.004$ compared to the polyculture). The specific growth rate was observed in the polyculture treatment, reaching 0.56% per day, followed by the lettuce and basil monoculture treatments at 0.42% and 0.29% per day, respectively, with no significant difference. Lastly, no significant effect was detected on the fin integrity of the perch across the treatment modalities.

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In terms of plants, at the end of the plant development, the polyculture treatment promoted a significant increase in the fresh aboveground and root biomass of lettuce compared to monoculture. Similarly, the rhizodeposits of lettuce in polyculture were significantly enriched in total nitrogen compared to monoculture. Conversely, chlorophyll content in lettuce leaves is significantly lower in polyculture than in monoculture. In basil, polyculture significantly increases anthocyanin content and significantly decreases flavonoid content in the leaves compared to monoculture.

Discussion & Prospects

The initial results of this experiment reveal a significant impact of aquaponic plant cultivation on perch performance, as well as on the plants' own development and physiology. The main hypotheses are a change in water composition and physicochemical properties induced by rhizodeposits, as well as a different pattern of aerial competition in the polyculture treatment. To supplement these results and provide a deeper understanding, numerous additional analyses are planned. These include an analysis of the perch's physiological and immune parameters, as well as water analyses to quantify its ecotoxicological impact, characterize its composition in terms of biomolecules, and bacterial composition.

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EFFECTS OF DIETARY VITAMIN A AND VITAMIN D₃ ON GROWTH PERFORMANCE AND PHYSIOLOGICAL RESPONSES OF PACIFIC WHITE SHRIMP *Penaeus vannamei*

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Introduction

Vitamins A and D are essential micronutrients involved in growth, epithelial integrity, immune regulation and metabolic homeostasis in aquatic animals. Vitamin D₃ exerts its biological effects through the vitamin D receptor (VDR), which forms a heterodimer with retinoid X receptor (RXR), suggesting that dietary vitamin A status may modulate physiological responses to vitamin D₃. Previous studies have shown that dietary vitamin D₃ can affect growth performance and physiological responses in *P. vannamei* (Dai et al., 2022; Lee and Lee, 2025). However, information on the combined effects of dietary vitamin A and vitamin D₃ in this species remains limited. Therefore, this study was designed to evaluate the interactive effects of dietary vitamin A and vitamin D₃ in juvenile *P. vannamei*.

Materials and Methods

A 2 × 4 factorial design was established with two dietary vitamin A levels (0 and 1.8 mg kg⁻¹) and four dietary vitamin D₃ levels (0, 30, 60 and 120 µg kg⁻¹), resulting in eight experimental diets designated as A0D0, A0D30, A0D60, A0D120, A1.8D0, A1.8D30, A1.8D60 and A1.8D120, respectively. Juvenile *P. vannamei* (initial body weight, 0.78 ± 0.01 g) were randomly distributed into 32 tanks (20 shrimp per tank), with four replicate tanks per treatment. An 8-week feeding trial is currently in progress. HPLC analysis determined dietary vitamin D₃ concentrations of 1.6, 37.0, 65.1, 121.6, 2.0, 39.7, 64.7 and 121.7 µg kg⁻¹, and dietary vitamin A concentrations of 0.23, 0.23, 0.23, 0.24, 2.00, 1.99, 2.01 and 2.00 mg kg⁻¹ corresponding to diets 1 to 8, respectively. Growth performance and survival are being monitored biweekly. At the end of the feeding trial, feed utilization, biochemical and physiological responses, digestive enzyme activity, innate immune responses, antioxidant capacity, histological parameters and gene expression related to vitamin A and vitamin D signaling, antioxidant defense, immunity and lipid metabolism will be evaluated.

Results

At both weeks 2 and 4, survival remained 100% across all treatments. At week 2, no significant effects of dietary vitamin A, vitamin D₃, or their interaction were observed for weight gain and specific growth rate ($P > 0.05$). At week 4, weight gain was significantly affected by dietary vitamin A ($P < 0.001$), vitamin D₃ ($P = 0.001$) and their interaction ($P = 0.018$). Similarly, specific growth rate was significantly affected by dietary vitamin A ($P < 0.001$), vitamin D₃ ($P = 0.001$) and their interaction ($P = 0.022$). Final results from the 8-week feeding trial will be further presented and discussed.

Conclusion

Dietary vitamin A, vitamin D₃ and their interaction significantly affected growth performance in juvenile *P. vannamei* at week 4. Completion of the feeding trial, along with subsequent physiological and molecular analyses, is expected to provide further insights into the nutritional roles and potential interactions of these two vitamins in *P. vannamei*.

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COMBINED EFFECTS OF ASTAXANTHIN AND AN EMULSIFIER ON GROWTH, LIPID UTILIZATION, IMMUNE RESPONSES, DIGESTIBILITY, INTESTINAL MICROBIOTA AND STRESS TOLERANCE IN PACIFIC WHITE SHRIMP *Penaeus vannamei*

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Introduction

P. vannamei is one of the most important aquaculture species worldwide, however, intensive culture conditions frequently expose shrimp to environmental stressors including salinity fluctuations and ammonia accumulation, which can compromise physiological homeostasis and reduce productivity. Nutritional strategies using functional feed additives have been extensively explored to mitigate these challenges. Astaxanthin (Ax), a lipophilic carotenoid, is known to improve growth performance, antioxidant capacity, immune responses, and stress tolerance. However, its efficacy may be constrained by limited bioavailability, potentially due to inefficient lipid digestion and emulsification in shrimp. Dietary emulsifiers (Em) have been suggested to enhance the absorption of lipophilic compounds and improve their utilization efficiency. Therefore, co-supplementation of Ax with an emulsifier may enhance its functional efficacy through improved bioavailability and potential synergistic interactions. Nevertheless, the combined effects of Ax and Em have not been systematically evaluated in *P. vannamei*. This study aimed to evaluate the interactive effects of dietary Ax and Em on physiological performance, immune response, antioxidant capacity, intestinal microbiota, and stress resistance under salinity and ammonia challenges in *P. vannamei*.

Materials and Methods

A basal diet (Con) was formulated without Ax and Em and four additional diets were prepared: 50 mg/kg Ax (A50), 25 mg/kg Ax + 350 mg/kg Em (A25E350), 50 mg/kg Ax + 350 mg/kg Em (A50E350) and 50 mg/kg Ax + 700 mg/kg Em (A50E700). Shrimp (initial body weight: 1.42 ± 0.01 g) were stocked into 25 tanks with 30 shrimp per tank in five replicates per dietary treatment and fed for 10 weeks. At the end of the feeding trial, growth performance and feed utilization were evaluated. Hemolymph, hepatopancreas, intestine and muscle samples were collected for analysis of immune responses (lysozyme, antiprotease, nitro blue tetrazolium and phenoloxidase), antioxidant capacity (superoxide dismutase, glutathione peroxidase, catalase and malondialdehyde), digestive enzyme activities (amylase, lipase, trypsin and chymotrypsin), gene expression related to antioxidant defense, pro-inflammatory and lipid metabolism, histological examination, Ax retention and meat quality (cooking loss, texture properties and color indices). Chromium oxide (Cr_2O_3 , 1%) was incorporated into the diets as an inert marker to determine the apparent digestibility coefficients (ADCs) of protein, lipid, fatty acids and energy. Intestinal microbiota was evaluated by quantifying total viable bacteria (TVB), lactic acid bacteria (LAB), *Vibrio* spp., and Gram-negative bacteria using selective culture media. For the salinity stress test, shrimp (18 shrimp per tank, triplicate) were exposed to low salinity (5 psu) for 2 h, followed by recovery to normal salinity condition (25 psu). Hemolymph was collected at 2, 24 and 48 h post-exposure for flow cytometry analysis. Flow cytometry was conducted to assess hemocyte count, cell mortality, nitric oxide and reactive oxygen species production, and phagocytic activity. For the ammonia challenge, shrimp (10 shrimp per tank, triplicate) were exposed to 2.8 mg/L ammonia, and survival was monitored for 60 h.

Results

Dietary supplementation of Ax significantly improved growth performance, feed efficiency, immune response and antioxidant capacity in shrimp, with additional improvements observed when combined with an Em. Co-supplementation (A50E350 and A50E700) resulted in improved growth and feed utilization efficiency, along with elevated high-density lipoprotein levels and reduced low-density lipoprotein levels. Co-supplementation of Ax and Em enhanced phenoloxidase, lysozyme, antiprotease and antioxidant enzymes activities, while reducing malondialdehyde levels. Em supplementation groups showed significantly improved lipase activity and ADCs of protein, lipid, energy and fatty acids. Gene expression analysis revealed the upregulation of genes related to antioxidant defense (catalase, glutathione peroxidase, superoxide dismutase) and lipid metabolism along with the downregulation of pro-inflammatory (I κ B kinase β , Tumor necrosis factor- α , Interleukin-16) and stress-related genes (heat shock protein 70) in co-supplemented groups. Ax concentration in muscle was significantly increased and further enhanced by Em, accompanied by improved meat quality, including

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increased hardness and enhanced coloration. Under salinity stress conditions, the A25E350 group showed significantly higher cumulative survival compared to the Con group. Under ammonia stress conditions, the A50, A25E350, A50E350, and A50E700 groups exhibited significantly higher cumulative survival than the Con group. Flow cytometry analysis showed that Em supplementation increased hemocyte counts and immune cell function activities while reducing hemocyte mortality, nitric oxide production and reactive oxygen species levels. Em supplementation favorably modulated intestinal microbiota as indicated by increased lactic acid bacteria and reduced *Vibrio* spp.

Conclusions

Co-supplementation of Ax and an Em effectively enhanced growth performance, physiological responses and stress resistance in *P. vannamei*. The improved outcomes were associated with enhanced immune function, antioxidant capacity, and nutrient utilization, along with increased Ax accumulation in muscle and improved meat quality. Notably, Em supplementation enhanced the functional efficacy of Ax, resulting in favorable modulation of intestinal microbiota, as well as improved stress tolerance and immune stability under salinity and ammonia challenges. Overall, these findings indicate that dietary Em may serve as an effective nutritional strategy to enhance the bioefficacy of Ax, providing a practical nutritional approach to improve productivity and robustness in shrimp culture.

DIETARY EMULSIFIER IMPROVES ASTAXANTHIN UTILIZATION UNDER REDUCED DIETARY LIPID CONDITIONS IN PACIFIC WHITE SHRIMP *Penaeus vannamei*

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Introduction

P. vannamei is a major aquaculture species in which dietary lipids play a critical role in supporting growth performance and physiological functions. Therefore, even moderate changes in dietary lipid levels may affect the digestion and utilization of lipophilic nutrients. Astaxanthin, a lipophilic carotenoid, has been reported to promote growth and physiological responses in shrimp, however, its efficacy is dependent on efficient lipid digestion and absorption. Dietary emulsifiers have been shown to improve lipid emulsification and facilitate the utilization of lipophilic compounds, suggesting a potential role in maintaining nutrient utilization efficiency under varying dietary lipid conditions. Therefore, co-supplementation of astaxanthin with an emulsifier may help sustain physiological performance under reduced dietary lipid levels. However, this interactive effect has not been clearly elucidated in *P. vannamei*. This study aimed to evaluate whether dietary astaxanthin and emulsifier can sustain growth performance and physiological responses under moderately reduced dietary lipid conditions in *P. vannamei*.

Materials and Methods

A control diet (Con, 8.4% lipid) was formulated without astaxanthin or emulsifier and two additional diets were prepared by supplementing 50 mg/kg astaxanthin without emulsifier (Ax50) and 50 mg/kg astaxanthin with 350 mg/kg emulsifier under reduced lipid conditions (LO-AxEm, 6.5% lipid). Shrimp (initial body weight: 1.42 ± 0.01 g) were randomly distributed into 15 tanks (30 shrimp per tank) with five replicates per treatment and fed experimental diets for 10 weeks. At the end of the trial, growth performance, feed utilization and apparent digestibility coefficients were evaluated. Hemolymph and tissue samples were collected (n=3 per tank) to assess immune responses, antioxidant capacity, digestive enzyme activity, gene expression, astaxanthin retention and meat quality (cooking loss, texture and color). Intestinal microbiota was analyzed by quantifying total viable bacteria, lactic acid bacteria, *Vibrio* spp., and Gram-negative bacteria using selective culture media. Stress tolerance was evaluated through salinity and ammonia challenge tests. For salinity stress, shrimp were exposed to low salinity (5 psu) for 2 h followed by recovery, and hemocyte-related responses were analyzed at multiple time points using flow cytometry. For the ammonia challenge, shrimp were exposed to 2.8 mg/L ammonia, and survival was monitored for 60 h.

Results

Compared to Con group, shrimp fed the Ax50 and LO-AxEm diets showed significantly improved growth performance and feed utilization, with no significant differences between these two treatments. Immune response and antioxidant capacity were enhanced in Ax50 group compared to the Con group, while the LO-AxEm group exhibited comparable levels to Ax50. Apparent digestibility coefficients of protein and fatty acids (C18:3n3, C20:5n3 and C22:6n3) were improved in both Ax50 and LO-AxEm groups compared to the Con group. Muscle Ax retention was significantly higher in both Ax50 and LO-AxEm groups compared to Con group. Meat quality improved in the LO-AxEm group, as indicated by higher redness (a*), yellowness (b*) and texture firmness, along with lower lightness (L*), compared to Con. Histological analysis revealed that lipid droplet accumulation and the abundance of F cells and B cells were significantly higher in LO-AxEm group than in Con group. Under salinity stress conditions, the Ax50 group showed significantly higher cumulative survival than Con and LO-AxEm groups, whereas under ammonia stress, both Ax50 and LO-AxEm groups exhibited higher cumulative survival compared to Con group. Flow cytometry analysis demonstrated enhanced hemocyte stability and immune cell function activities in both Ax50 and LO-AxEm groups compared to Con group, with comparable responses between the two treatments. In addition, intestinal microbiota was favorably modulated in LO-AxEm group, as indicated by increased lactic acid bacteria and reduced *Vibrio* spp. compared to both Con and Ax50 groups.

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Conclusions

Co-supplementation of astaxanthin and emulsifier effectively maintained growth performance, nutrient utilization, and physiological responses under reduced dietary lipid levels (6.5% crude lipid) in *P. vannamei*. The LO-AxEm group exhibited comparable performance to Ax50 for growth, immune response, antioxidant capacity, digestibility, and hemocyte-related functional responses. Notably, emulsifier co-supplementation further enhanced product quality and intestinal health, as evidenced by improved meat quality, favorable modulation of intestinal microbiota and increased lipid droplet accumulation and digestive cell (F cells and B cells) abundance. Overall, these findings suggest that co-supplementation of astaxanthin and emulsifier may represent an effective nutritional strategy to maintain performance and improve product quality under reduced dietary lipid levels in shrimp culture.

GENOMIC SELECTION FOR GROWTH TRAITS IN OLIVE FLOUNDER UNDER EXTRUDED PELLET FEEDING REGIMES WITH CONTRASTING FISHMEAL LEVELS

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Introduction and Methodology

Olive flounder (*Paralichthys olivaceus*) is the dominant marine aquaculture species in South Korea, yet its production system faces increasing challenges due to reliance on moisture pellets (MP), which depend on limited fishery resources and pose sustainability concerns. Although extruded pellets (EP) provide practical and environmental advantages, their widespread adoption has been hindered by reduced growth performance. Improving growth under EP-based feeding systems is therefore a critical objective for sustainable aquaculture. In this study, we investigated the genetic architecture of growth traits and evaluated genomic prediction strategies under two EP-based diets differing in fishmeal content: a commercial diet and a low-fishmeal diet. A large-scale population was established, and individuals were repeatedly phenotyped for growth traits, including body weight (BW), weight gain rate (WGR), and specific growth rate (SGR), and genotyped using a high-density SNP array.

Results and Discussion

Genome-wide association analysis revealed that growth traits were predominantly polygenic under both dietary conditions, with only a single SNP reaching genome-wide significance under the low-fishmeal diet. Heritability estimates indicated that BW consistently exhibited moderate to high heritability, whereas WGR and SGR showed relatively lower values. These results highlight BW as the most reliable trait for genomic improvement. Genomic prediction analyses demonstrated that predictive performance varied across traits and diet groups, with BW achieving the highest accuracy. Notably, incorporating informative SNPs identified through GWAS markedly improved prediction accuracy, with values approaching 0.8. Evaluation of genotype-by-diet interaction showed high genetic correlations for BW across diets, indicating stable genetic ranking and supporting the use of a common prediction model. In contrast, WGR and SGR exhibited evidence of diet-dependent genetic responses, suggesting the need for trait-specific modeling strategies. Genomic prediction analyses demonstrated that predictive performance varied across traits and diet groups, with BW achieving the highest accuracy. Notably, incorporating informative SNPs identified through GWAS markedly improved prediction accuracy, with values approaching 0.8. Evaluation of genotype-by-diet interaction showed high genetic correlations for BW across diets, indicating stable genetic ranking and supporting the use of a common prediction model. In contrast, WGR and SGR exhibited evidence of diet-dependent genetic responses, suggesting the need for trait-specific modeling strategies. Based on genomic estimated breeding values, superior broodstock were selected to establish an elite F1 population, enabling the practical application of genomic selection in breeding programs. Ongoing data collection from this population will further enhance model accuracy and robustness. Overall, this study demonstrates that genomic selection can effectively improve growth performance under varying feed conditions and provides a practical framework for developing feed-adaptive breeding strategies in olive flounder aquaculture.

SALINITY-DEPENDENT REMODELING OF GILL IONOCYTES AND ITS IMPACT ON ANTIVIRAL RESPONSES TO NERVOUS NECROSIS VIRUS IN *Epinephelus lanceolatus*

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The giant grouper (*Epinephelus lanceolatus*) is a euryhaline aquaculture species capable of tolerating salinities ranging from freshwater (0‰) to hypersaline environments (50‰). Despite its remarkable salinity tolerance, the cellular mechanisms underlying this physiological plasticity, and their interaction with antiviral defense, remain incompletely understood. This study examined gill ionocyte morphology, distribution, and Na⁺/K⁺-ATPase (NKA) responses in juvenile giant groupers acclimated to five salinity conditions (0‰, 5‰, 10‰, 35‰, and 50‰), and further evaluated their responses following infection with nervous necrosis virus (NNV). Following NNV challenge, antiviral responses displayed salinity-dependent modulation, with fish maintained near seawater conditions (35‰) exhibiting more robust antiviral capacity, whereas extreme salinities were associated with altered host responses and susceptibility to viral infection. Despite substantial gill remodeling, muscle water content and plasma osmolality remained stable across treatments, indicating effective systemic osmoregulatory compensation. Collectively, these findings reveal coordinated structural and molecular plasticity of the grouper gill in response to salinity variation and demonstrate that environmental salinity critically shapes host–virus interactions. This study highlights the role of NKA-mediated ionocyte regulation as a key mechanism supporting extreme euryhalinity, while also uncovering a functional trade-off between osmoregulation and antiviral defense. These insights provide a mechanistic basis for optimizing salinity conditions to improve disease resistance and inform salinity-flexible aquaculture strategies.

HIGH CO₂ IN THE WATER CAUSES NEPHROCALCINOSIS IN JUVENILE RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Nephrocalcinosis continues to be one of the most important production disorders in the production of Atlantic salmon and rainbow trout (Moldal et al 2025). Nephrocalcinosis is multifactorial, and amongst other causes associated with factors such as high fish densities and suboptimal water quality. A classical long-term experiment by Smart et al (1979) showed strong correlation between high CO₂ in water and nephrocalcinosis in rainbow trout. However, these results have not been confirmed in later studies with rainbow trout (60-900 g) in RAS (Good et al., 2010). Mota et al. (2019) exposed A. salmon to six dissolved CO₂ concentration ranging from 5-40 mg CO₂ l⁻¹ over a 12-week period. Except for skin they found no major effect of health and welfare, including nephrocalcinosis. The causes of nephrocalcinosis in A. salmon seem to be more complex than in rainbow trout. The aim of this study was to confirm that CO₂ as the only environmental variable leads to the development of nephrocalcinosis in rainbow trout.

Material and Methods

All female rainbow trout juveniles were exposed to 5 different levels of added CO₂ (nominal 0, 10, 15, 20 and 25 mg CO₂ l⁻¹) in the inlet water for 7 weeks. Actual CO₂ concentrations were 7.3, 11.9, 16.7, 22.1 and 26.3 mg CO₂ l⁻¹. 200 juvenile rainbow trout (15 g) were distributed to each of 15 tanks; volume 500 l. Temperature 12°C. Two holding tanks were used to obtain the different CO₂ concentrations. Only O₂ was added to one tank, in the other one O₂, CO₂ and sodium bicarbonate as buffer. An Oxyguard Pacific unit was used to monitor and regulate CO₂ and pH in this tank. The setpoints in the holding tank for CO₂ dosing was 25 mg CO₂ l⁻¹ and pH 6.9, and dosing of CO₂ and buffer was regulated automatically. In the tanks, different CO₂ levels were adjusted by proportional mixing of water from both holding tanks (0 and 25 mg CO₂ l⁻¹). Buffer addition allowed us to keep tank pH equal between treatments, independent of CO₂ treatments. CO₂-addition was stopped after 8 weeks. Sampling of fish for weight, kidney and skin histology and high-resolution radiography was done before start of the experiment, week 5 and week 8. Radiography and histology were used for documentation of nephrocalcinosis. Radiograph images of kidneys were scored on a range of 0-4.

Results

There was a strong correlation between added CO₂ and prevalence of nephrocalcinosis; zero in the control group (no CO₂ added) increasing to 41,7% in the 25% mg CO₂ l⁻¹ group. The severity of nephrocalcinosis increased with increasing addition of CO₂. The growth of the fish was also correlated with CO₂ levels. At end of the experiment the mean weight of the control group was 89.2 g and 72.6 for highest CO₂ group. There was little visible effect on skin quality at 50 g size, but clear changes were observed at the end of the experiment, suggesting long-term effects. The findings on skin histology indicate increased stress, weaker skin structure and thinner epidermis in the group that received 25 mg CO₂ l⁻¹ compared to the control group without the addition of CO₂. Results from the kidney histology evaluation will be presented later.

Conclusions

The experimental setup offered very good control of the water quality parameters O₂, CO₂ and pH. We found a very clear correlation between high CO₂ and prevalence of nephrocalcinosis in rainbow trout. High resolution radiography seems to be an excellent tool to diagnose active nephrocalcinosis. Both growth and skin were negatively affected by increasing CO₂ levels. The skin is an important barrier against infectious agents in fish, and suboptimal skin quality will increase the risk of wounds and infections. The results from this experiment suggest that the relationship between high CO₂ in the water and the development of nephrocalcinosis is much stronger in rainbow trout than in salmon, which appears to have a more complex causal mechanism.

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TOWARDS INTEGRATED LIFE CYCLE SUSTAINABILITY AND CIRCULARITY ASSESSMENT FOR EMERGING AQUACULTURE SYSTEMS: INSIGHTS FROM INNOAQUA

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Life cycle assessment (LCA) is a key tool for evaluating the environmental performance of bio-based systems, yet important challenges remain in emerging aquaculture systems characterised by early-stage technologies, multifunctionality, and complex valorisation pathways. In parallel, circularity assessment is gaining relevance, but its integration within life cycle-based sustainability frameworks is not well established.

This work presents the methodological framework developed in the INNOAQUA project to advance the integration of circularity into Life Cycle Sustainability Assessment (LCSA) for aquaculture systems. The approach supports consistent and decision-relevant sustainability evaluation across multiple innovations, including algae-based ingredients, fish by-product valorisation routes, and bio-based products.

The methodology is structured around a dual-level assessment. At ingredient and product level, a four-step framework is applied: system structuring through harmonised Value Stream Maps, circularity assessment based on water recirculation, waste minimisation, waste valorisation and material circularity indicators, environmental assessment using LCA, and integration of sustainability and circularity dimensions. At project level, environmental impacts are assessed through scenario-based aggregation of life cycle results, while circularity is evaluated through a portfolio based interpretation, reflecting its non-additive nature.

The framework highlights key methodological challenges in aligning circularity with life cycle-based sustainability assessment. Circularity indicators describe intrinsic system properties that do not scale linearly and cannot be directly aggregated, limiting their integration into conventional LCSA approaches. In addition, trade-offs between circularity strategies and environmental performance may arise, particularly in processes requiring increased energy or processing intensity.

These findings support positioning circularity as a complementary dimension within LCSA rather than a directly aggregable metric. The INNOAQUA framework contributes to efforts to harmonise life cycle approaches for emerging bio-based systems by providing a structured and scalable methodology. The insights generated are transferable to other aquatic and bio-based value chains, supporting more robust and decision-relevant sustainability evaluations.

Disclaimer/ Disclosure

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ASSESSMENT AND APPLICATION OF A FUNCTIONAL TRAIT-BASED APPROACH FOR DESIGNING POLYCULTURE WITH MINIMAL TROPHIC COMPETITION

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Introduction

Polyculture, defined as the simultaneous rearing of multiple fish species within the same system, tends to be most efficient when co-occurring species exploit distinct trophic resources. A key challenge is therefore to minimize trophic competition, which can otherwise reduce growth through detrimental interactions and suboptimal resource use. The intensity of trophic competition can be estimated from the trophic distance between species, based on trophic functional traits reported in the literature (Lecocq et al., 2024). However, distances based on trophic trait proxies have not yet been compared to realized trophic interactions under field conditions. The reliability of the trophic trait approach therefore remains to be established using methods capable of quantifying such interactions in experimental conditions such as stable isotope analysis.

The objective of this study was therefore to assess whether trophic distances derived from species trophic functional traits are consistent with those obtained from stable isotope mixing models (SIMM), to determine the reliability of the functional trait-based approach as a proxy for trophic competition in polyculture systems.

Method

The assessment was conducted in two ponds containing both stocked and wild fish species, alongside cage-fed tilapia. Distances were then computed using standardized matrices. For the functional trait approach, the matrix reflected the relative contributions of different trophic functional groups selected from the FishBase classification. For the stable isotope approach, fish muscle and multiple potential food sources (aquatic macrophytes, insects, crustaceans, snails, fish, soil and suspended organic matter, biofilm, and commercial pellets for tilapia) were sampled in both ponds and analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to estimate source contributions to fish species diet. The standardized matrix was subsequently constructed from the normalized mean contributions of these sources, as inferred using the SIMM. The Bray–Curtis distance was used as the metric for both approaches. The performance of the functional trait approach was assessed by comparing the distance matrix derived from trophic traits-based approach with that obtained from the SIMM, using a Mantel test.

Results and discussion

In both ponds, significant positive correlations ($r^2 > 0.5$; $p\text{-value} < 0.005$) were detected between distances derived from the trophic trait-based approach and those obtained from the SIMM. These results suggest that the functional trait-based method can be accurate when species diets are well documented. However, substantial discrepancies occurred for some species, notably due to pellet consumption from cages and context-dependent feeding behavior which can be influenced by resource availability, density, and individual preferences. Overall, we showed that this functional trait-based approach offers a rapid, cost-effective and practical framework for selecting fish assemblages with minimal trophic competition to improve polyculture design. Based on this, we further illustrated an application of the trophic trait-based approach to rank combinations drawn from a larger candidate pool within the two study ponds.

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PREDISH: A MODEL FOR ESTIMATING RISK OF FISH PREDATION IN AQUACULTURE SYSTEMS

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Background and objective:

Fish polyculture is an age-old practice that remains widely applied across most continents (Tchiedjo et al., 2026). It refers to the simultaneous rearing of multiple fish species within the same undivided physical space of an aquaculture system, with the aim of producing economically valuable biomass (Stickney, 2013). It offers numerous benefits for enhancing the sustainability of aquaculture, provided the species are compatible (i.e., able to coexist without detrimental interactions) (Thomas et al., 2021).

Among detrimental interactions, predation (defined here as the complete ingestion of a living prey) is the most critical to avoid in polycultures (Lecocq et al., 2024). However, testing every possible species combination to identify those free of predation is impractical and ethically problematic. To address this, we present Predish: a model that estimates predation risk between fish using only total length (i.e., the distance from the tip of the snout to the end of the caudal fin) as input, allowing therefore to avoid empirical trials.

Materials and methods:

This model is adapted from niche allometric framework and was calibrated using an extensive dataset of fish predation where the total length of the piscivore and its prey are known. The model was validated using the specificity (non-predation prediction accuracy), the sensitivity (predation prediction accuracy), the True Skill Statistic (TSS) and the area under the curve (AUC) method using a comprehensive validation dataset of fish predation (2,101 instances) and non-predation events (13,707 instances).

Conclusion:

The model performances were considered as good with a sensitivity of 0.88, specificity of 0.96, a TSS of 0.84, and an AUC of 0.92. This model is therefore a valuable resource in order to designing new polycultures with minimal predation risk to support the development of more sustainable aquaculture.

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MEDIUM CHAIN FATTY ACIDS-RICH OIL IN RAINBOW TROUT DIETS: GROWTH AND DIGESTIBILITY ALONG THE DIGESTIVE TRACT

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Introduction

The expansion of aquaculture raises sustainability concerns due to its reliance on finite marine resources, driving aquafeed industry to seek alternative efficient lipid sources aligned with a circular bioeconomy. Medium-chain fatty acids (MCFAs; 6-12 carbons) have shown potential benefits in fish nutrition as they are highly soluble and rapidly oxidized for energy (Liu et al 2025). The available MCFAs sources ($\approx 40\%$ of total fatty acids) derived from plant oils (e.g. palm kernel oil and palm kernel fatty acid distillate, a by-product of crude palm kernel oil refining, with more than 60 % of free fatty acids (FFA) or insects such as the black soldier fly. When evaluating new feed ingredients, assessing their digestibility and their effect on growth performance are key steps. Although faecal digestibility overall provides useful estimates of lipid utilization, understand the fatty acid (FA) absorption dynamics along the gastrointestinal tract (GIT) can give deeper insight into bioavailability and lipid efficiency. However, such information related to MCFAs is limited, particularly beyond Atlantic salmon (Røsjø et al 2000). This study investigated different MCFAs-rich lipid sources in diets for rainbow trout (*Oncorhynchus mykiss*), focusing on the FA absorption dynamics along the digestive tract.

Materials and Methods

The study was conducted at the Experimental Facility of DISAFA, University of Turin (Italy). Four isonitrogenous (38.5% CP) and isolipidic (25% EE) extruded diets were formulated: a Control (CON) diet containing 7% fish oil and 15% rapeseed oil (RO), and three test diets in which 50% of the RO was replaced with different MCFA-rich oils: Black soldier fly oil (BSFO), palm kernel oil (PKO), and palm kernel fatty acid distillate (PKFAD). Two trials using rainbow trout were performed: a) 18-week growth trial (12 tanks, 32 fish/tank, 3 replicates/diet), and b) 8-week digestibility trial (12 tanks, 20 fish/tank, 3 replicates/diet). Faeces were collected according Choubert method (1982). Apparent digestibility coefficients (ADCs) were assessed using yttrium oxide (0.1 % of the diet) as inert marker. At the end the experimental period, the content of stomach, pyloric caeca (PC), anterior (AI) and posterior intestine (PI) from 5 fish/tank were collected and stored at -20°C for analysis.

Results

Partial replacement of RO by MCFA-rich oils significantly affected final body weight (FBW). Fish fed the BSFO diet showed the highest FBW (741.69 g), followed by PKFAD diet (713.65 g), significantly higher than CON and PKO diets (689.67g and 673.94g; $P \leq 0.001$). No differences were found in feed conversion ratio, daily feed intake and daily weight gain and specific growth rate among treatments ($P > 0.05$). The FA digestibility varied along the GIT. In the stomach, all the FAs showed negative ADC values across all diets. In the PC, total FA (TFA) digestibility was $\sim 60\%$, with differences only in MCFAs among treatments: PKFAD showed higher ADC (71.53%) than PKO (57.55%) and BSFO diets (53.68%) ($P < 0.01$). Except for PKFAD, these values are lower than for monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). In the AI, TFA digestibility increased to 85–87%. MCFA digestibility was higher in MCFA-rich diets ($\sim 91\%$) than in CON (46.92%) ($P < 0.01$), reaching levels similar to MUFA and PUFAs for all experimental diets. Digestibility of long-chain saturated FA (LCSFAs, $\geq 14\text{C}$) was lower in PKFAD diet (71.75%) than in BSO (80.22%) and

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PKO (80.25) ($P=0.022$). In the PI, TFA digestibility was higher in CON fish (93.32%) than in PKFAD (88.24%) ($P < 0.05$). No significant differences were observed among MCFA-rich diets for MCFAs and PUFAs. In faeces, TFA digestibility reached the 95–97 %, highest in CON diet (98.28 %) and the lowest for PKFAD (95.24%) ($P < 0.01$). Overall, the PC region constitute the main absorption site for all FA (64–69% of total absorption for TFA). However, its contribution was greater in PKFAD diet for all FAs, mainly MCFAs (79 %), but not for PUFA.

Discussion

This study shows that the dietary inclusion of 7% of MCFA-rich oils supports growth in rainbow trout comparable to, or improved, than the control diet, suggesting that these oils can provide an adequate energetic value similar to RO. Unlike previous results (Verge-Mérida et al., 2022), high FFA content did not impair growth, as shown by the higher FBW in PKFAD-fed fish versus PKO and CON. However, growth differences were not consistently reflected in digestibility results. As reported in Atlantic salmon (Røsjø et al., 2000), the PC is the main site for FA absorption, especially in FFA-rich diets, although the AI also contributed substantially (~35%). The final lower overall digestibility in PKFAD diet, will be related to its high FFA content and reduced absorption of LCSFA present in the diet, particularly from the AI onwards, linked to their hydrophobicity, high melting point and greater tendency to form insoluble soaps that limits their absorption (Verge-Mérida et al. 2022). In relation to MCFAs, the higher digestibility for PKFAD diet than for BSO and PKO diets in PC disappear in the AI, where ADC values were similar to PUFA. The shorter chain length of MCFAs enhances solubility and allows more direct intestinal absorption without or with less dependence on bile-mediated micelle formation (Çenesiz and Çiftci, 2020), being more evident and faster when present as FFA. Overall, this study confirms the potential of MCFA-rich oil as viable energy source in trout diets and highlight distinct FA absorption patterns along the GIT.

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GLOBAL AQUACULTURE GROWTH: SUSTAINABLE INTENSIFICATION AND EXPANSION?

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Introduction

With capture fisheries development at a standstill (Parker et al., 2018), aquaculture has become a key sustainable pathway for meeting the continuously growing demand for aquatic products and strengthening the resilience of food systems (Troell et al., 2014). We explore how current interconnections between the aquaculture, crop, livestock, and fisheries sectors act as an impediment to, or an opportunity for, enhanced resilience in the global food system given increased resource scarcity and climate change. Aquaculture can potentially enhance resilience through improved resource use efficiencies and increased diversification of farmed species, locales of production, and feeding strategies. However, aquaculture's reliance on terrestrial crops and wild fish for feeds, its dependence on freshwater and land for culture sites, and its broad array of environmental impacts diminishes its ability to add resilience. Feeds for livestock and farmed fish that are fed rely largely on the same crops, although the fraction destined for aquaculture is presently small (~4%). Two widely discussed pathways for future aquaculture growth, spatial expansion and intensification, both face ecological and resource constraints, giving rise to environmental impacts and even public health risks (Caputo et al., 2023; Ottinger et al., 2016). A key question emerges: should future growth rely on more area, more input intensity, or higher productivity? This study integrates global data on aquaculture area, labor, feed use, production, and output value to examine efficiency patterns, their determinants, and the sources of output growth. The findings point to a viable development pathway for aquaculture and provide a scientific basis for formulating differentiated development strategies for the aquaculture industry.

Materials and Methods

This study integrated global data on aquaculture area, labor, feed, fishmeal/fish oil inputs, production, output value, species-level environmental footprints, and country-level socioeconomic and biophysical indicators, classified by species group, production system, and development context. Production efficiency was assessed via area- and labor-based productivity, feed conversion ratio, and fishmeal/fish oil intensity. Temporal trends and cross-system differences were analyzed using descriptive and nonparametric statistics, and productivity-environment trade-offs were evaluated using standardized indicators against observed development patterns. Bayesian bootstrap correlation and random forest models identified factors associated with efficiency, and a Cobb-Douglas growth accounting framework decomposed output growth into area expansion, input intensification, and total factor productivity.

Results

Global aquaculture area and employment plateaued after the mid-2010s, while area- and labor-based productivity rose substantially. Despite reduced fishmeal and fish oil use intensity, feed conversion efficiency declined as the share of fed species in total production increased. Efficiency varied systematically across species, techniques, environments, continents, and income groups, with physical-output and economic-value rankings often diverging. Output growth aligned more closely with value-based productivity than environmental performance. Socioeconomic development (e.g., GDP, electricity) was broadly linked to higher productivity, but area-based efficiency was primarily shaped by culture technique, while labor-based efficiency depended more on continental and environmental context. Growth accounting revealed a shift from area expansion toward input intensification and, increasingly, TFP growth, which now accounts for nearly half of output growth.

Discussion

Despite current optimism about aquaculture's expansion potential, our findings indicate that future growth potential increasingly depends on sustained total factor productivity (TFP) growth rather than spatial expansion or increased input use. Environmental performance alone is insufficient for long-term competitiveness, which instead depends on converting area and labor into economic value under resource constraints. We advocate a land-sparing pathway of TFP-driven sustainable intensification that reduces ecosystem pressure while supporting biodiversity and climate goals, consistent with FAO's Blue Transformation. Given that efficiency and its drivers vary across regions and production systems, such intensification should be pursued through context-specific, differentiated policies.

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BIOBOOST-PRO: DEMONSTRATING AND ENABLING BIOPONICS USING MANURE-BASED ORGANIC FERTILIZERS IN NORTH-WEST EUROPE, WITH A FOCUS ON FISH SLUDGE VALORISATION

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Hydroponic crop production is increasingly adopted in North-West Europe (NWE) due to its high productivity, efficient water use and reduced dependence on soil quality. However, these systems remain largely reliant on petrochemical mineral fertilisers, whose production is energy-intensive and based on finite resources. At the same time, large amounts of organic residues and manures, including fish sludge from aquaculture and aquaponic systems, are produced across NWE, generating environmental pressures and nutrient losses when not adequately valorised. In this context, fish sludge represents a particularly relevant resource for the aquaculture sector, as it contains recoverable nutrients that could contribute to more circular and resource-efficient production systems.

BIOBOOST-PRO (Interreg North-West Europe 2021–2027) addresses this dual challenge by promoting biaponics, defined as hydroponic crop production using organic fertilisers derived from organic matter sources. The project aims to demonstrate the technical feasibility, agronomic performance and scalability of bioponic systems based on animal manure, while lowering the barriers to their adoption by growers and stakeholders across the value chain. Within this broader framework, particular attention is given to the valorisation of fish sludge, as it creates a direct link between aquaculture waste management and greenhouse crop production, and illustrates how aquaculture by-products can be reintegrated into productive circular systems.

Materials and Methods

The project is structured around three complementary work packages: (i) showcasing biaponics in real greenhouse conditions, (ii) developing economic, strategic and practical tools to enable replication, and (iii) engaging stakeholders to stimulate long-term uptake. BIOBOOST-PRO is implemented across seven pilot locations representing eleven bioponic set-ups in Belgium, France, Germany, Ireland and the Netherlands.

The pilot systems differ in manure source (fish, poultry and pig), crop type (lettuce, basil, tomato, herbs and ornamental plants), and system configuration. Both on-site systems, where digestion and crop production are located at the same site, and off-site systems, where digestate is transported from an external digester to the greenhouse, are investigated. This diversity allows the assessment of biaponics under contrasting technical, economic and territorial contexts representative of NWE. Among these systems, fish-based set-ups are of specific interest because they explore the conversion of aquaculture sludge into plant-available nutrients and therefore provide a concrete pathway for integrating aquaculture residues into soilless crop production.

A jointly developed transnational measurement plan ensures harmonised monitoring across all pilots. Key parameters include plant growth and yield, nutrient dynamics (NH_4^+ , NO_3^-), pH, dissolved oxygen, water and air temperature, CO_2 concentration, and system input–output flows. Data are collected using a shared transfer system to enable cross-site comparison, modelling and optimisation. In the fish-based systems, this monitoring is particularly important to assess nutrient release from sludge, nitrification efficiency, nutrient balance, and the technical constraints associated with residual organic matter in bioponic cultivation.

Results and Discussion

As experimental trials are ongoing, this contribution focuses on the expected outcomes and first scientific contributions of BIOBOOST-PRO. From an agronomic perspective, biaponics is expected to maintain crop yield and quality comparable to conventional hydroponics, while improving nutrient recycling efficiency through the use of organic fertilisers. In particular, fish-sludge-based fertilisation is expected to demonstrate how aquaculture-derived nutrients can be recovered and reused in plant production, thereby reducing nutrient discharge and supporting circularity between aquaculture and horticulture.

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At the system level, BIOBOOST-PRO aims to demonstrate that digestates from fish, poultry and pig manure can partially or fully substitute petrochemical fertilisers in greenhouse hydroponics. The comparison between on-site and off-site configurations will help identify context-specific advantages, constraints and scalability potential. For fish sludge, this comparison is especially relevant, as nutrient recovery may be integrated directly on-site in decoupled aquaponic or aquaculture-linked systems, or implemented through external treatment and transfer to greenhouse units. In addition, the integration of organic biostimulants is expected to further enhance nutrient uptake and crop resilience.

Beyond technical performance, a major innovation of BIOBOOST-PRO lies in its integrated approach to manure valorisation, linking experimental bioponic systems with waste management, territorial planning and stakeholder engagement. The project develops transnational tools to identify technical, logistical and legislative barriers to the use of manure-derived digestates in hydroponic systems, and to assess their economic and regulatory feasibility across North-West Europe. By quantifying nutrient flows from fish, poultry and pig manure at regional scale and comparing them with the nutrient demand of greenhouse bioponic production, BIOBOOST-PRO evaluates the potential contribution of bioponics to closing nutrient loops and reducing nutrient losses from organic waste streams. In the case of fish sludge, this approach is particularly relevant for aquaculture, where sludge management remains a key environmental and economic challenge, and where nutrient recovery could become an additional lever for system resilience and sustainability.

To translate these insights into practice, BIOBOOST-PRO combines a hands-on implementation toolkit, co-created business models and a methodology for territorial strategy development, tested in seven pilot regions. Uptake is further stimulated through learning networks for growers and manure producers, transnational advocacy activities targeting policymakers and umbrella organisations, and broader awareness-raising actions. This integrated approach aims to support the roll-out of bioponics as a circular waste valorisation pathway, including for aquaculture-derived residues such as fish sludge, and thereby contribute to more resilient and resource-efficient food production systems in North-West Europe.



Figure 1 : Basil grown under six conditions, fertilized with fish digestate sludge, mineral fertilizer, and organic fertilizer

DISSEMINATION INCREASES THE VALUE OF RESEARCH SUBTITLE: TRANSFORMING RESEARCH COMMUNICATION WITHIN THE NORWEGIAN SEAFOOD RESEARCH FUND (FHF)

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Background The Norwegian Seafood Research Fund (FHF) finances research in Europe's leading aquaculture nation. Funded by a 0.3% statutory levy on the Norwegian seafood industry, there is significant stakeholder interest in the strategic allocation of these funds and their subsequent benefit to the sector. In 2024, the FHF Board of Directors mandated an enhanced strategic focus on dissemination. The directive required direct communication with the industry to facilitate the swift implementation of new knowledge as projects are completed. This presentation outlines how FHF has restructured and modernised its communication framework to bridge the gap between researchers, industry stakeholders, and policymakers.

Method and Strategy To address the diverse requirements of various target audiences, FHF transitioned to a proactive, bespoke communication model characterised by targeted initiatives for specific themes and individual projects. Moving away from a "one-size-fits-all" approach, channels and formats are now selected according to their specific objective: short video clips and social media are employed to build public awareness and trust; trade media and seminars are utilised to ensure the adoption of knowledge within the industry; and dedicated policy briefs are produced to inform policy development and regulatory frameworks.

Furthermore, FHF has established robust routines to monitor research projects from inception to completion, providing coordinated communication support that enables researchers to disseminate their findings rapidly and effectively. To ensure digital platforms successfully simplify scientific content for the end-user, Gerry McGovern's "Top Task" methodology was implemented. This approach prioritises the essential tasks users wish to perform and optimises their completion rates.

The communications department operates according to several Key Performance Indicators (KPIs) set by the Board. Following the successful results achieved in 2025, more stringent KPIs have been introduced, necessitating an even more systematic approach to communication. With a core team of three employees, FHF relies on a network of external specialists across various disciplines, including cinematography, photography, animation, social media, design, and event management. This presentation will detail how we collaborate with external providers to achieve our overarching strategic goals - the primary objective being to reach the seafood industry with new knowledge.

The presentation will also provide concrete examples of targeted communication for different audiences. We will demonstrate how reputation surveys are utilised to refine dissemination strategies, ensuring information is delivered via the channels most preferred by the industry. Furthermore, we will emphasise the strategic advantage of prioritising proactive external dissemination over internal administrative tasks.

Innovation and Future Prospects As digital search behaviours evolve, FHF is shifting its focus from traditional Search Engine Optimisation (SEO) to Answer Engine Optimisation (AEO). We strategically analyse how our research ranks within Google's AI-generated overviews and actively integrate AI chatbots and AI-driven English translation to enhance the global visibility and accessibility of Norwegian aquaculture research.

Conclusion By professionalising external communication through targeted formats and embracing artificial intelligence and user-centred web design, FHF ensures that research funding is converted into tangible, high-value knowledge. Dissemination is fundamental to increasing the value of research. This presentation shares practical insights and examples, offering a model for modernising research communication that is highly relevant to the wider European aquaculture sector.

MORPHOLOGICAL DEVIATIONS AND HISTOPATHOLOGY OF HEARTS OF RAINBOW TROUT (*Oncorhynchus mykiss*) REARED IN FINLAND

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Introduction

Compromised cardiac health negatively impacts the welfare of fish and could also lead to pre-slaughter losses. Pathogenic infections such as cardio myopathy syndrome (CMS) and non-infectious factors, such as deformities can both lead to adverse cardiac outcomes. The altered morphology such as round ventricles and misalignment of bulbus, that occur more often in farmed salmon than wild ones (Poppe et al. 2003), could be also associated with decreased cardiac performance and reduced stress resistance resulting in arrhythmias and cardiac episodes (Brijs et al. 2020). Farmed salmonids also have high prevalence of atherosclerosis, which can lead to lower oxygenation of cardiac cells during stressful events requiring higher cardiac performance (Brijs et al. 2020). Rearing practices promoting fast growth could increase the severity of cardiac deviations and coronary lesions (Frisk et al. 2020). However, there is still lack of knowledge of factors contributing in formation of morphological deformities.

Visual classification has been proposed as a superior tool for describing morphological deviations compared to commonly used morphometric ratios, which are not able to describe vast diversity of morphological variations (Engdal et al 2024). In this study we applied visual classification method for rainbow trout along with morphometrics and histology to get a comprehensive overview of heart health of commercially farmed slaughter sized rainbow trout in Finland. Our aim was to get an overview of cardiac deviations, prevalence and severity of coronary atherosclerosis, cardiac injuries and infections and to compare the differences between different farming sites and the origins of fish.

Materials and methods

Altogether 180 hearts of slaughter size rainbow trouts were collected and fixed in formalin from three net cage farms having fish of three origins. Two commercially reared breeding lines were also included in samples. Hearts were photographed following protocol by Engdal et al. (2024). Six hearts from each sampling group were randomly selected for histology. Bulbuses of these hearts were cut in to three cross sections and ventricles cut half sagittally. Visual classification of morphological deviations was done according by Engdal et al.'s (2024) protocol established for Atlantic salmon. The presence of deviation was marked either as present or absent. Morphometrical measurements were analysed with ImageJ according Poppe (2003) and Brijs (2020). Coronary lesion severity was scored on a scale 0-5 with small modification on Brijs et al. (2020) protocol. Sections of bulbuses and ventricles were examined for having epicarditis, endocarditis and signs of cardiac injuries. Histopathological findings were classified either present or absent.

Results

Out of 43 different deviation categories average amount of deviations per heart was 10 (range 2-19). Number of all morphological deformities did not differ between origin or farming site, nor did it correlate with body mass or relative heart size. However, certain categories had significant differences between farming environment and origin of fish. Most common ventricle deviations were low left dorsal apex (47 %) along with extended right dorsal ventricular apex (40 %), therefore hearts appeared in major part asymmetrical. 38 % of right ventricular sides were also distinctly enlarged compared to left side. 38 % hearts had ventroventricular ridge curving inwards, which has been previously seen in higher prevalence in moribund and dead fish (Engdal et al 2024). From deviations of bulbus most common findings were ballooning and bulging bulbus (42 %) and misaligned bulbus (40 %). Extensive fat was present in 26 % of hearts. Prevalence of extensive fattiness did not differ among origin or farming site. From histology samples 93 % fish had coronary lesions. Severity of atherosclerosis did not differ between origin of hatchery or farming site. Epicarditis was detected in 46 % and endocarditis in 32 % of bulbus samples. Significantly more fish from lake farm had epicardial (76%) inflammation. All of the ventricles had signs of recent hypoxic cardiac injury.

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Discussion

All the analysed hearts had morphological deviations, therefore altered morphology seems to be more rule than exception. Distinct finding of our study was high prevalence of asymmetrical shape of ventricles. The asymmetry of ventricle has previously been associated with impaired cardiac performance. Previous methods measuring symmetry were not sufficient, since symmetry axis is commonly measured from the left lateral projection, which has usually been extended side in Atlantic salmon. In our study right side of ventricle was found to be extended and enlarged one, usually combined with low left dorso-ventricular apex, which narrows down the symmetry angle and thus seemingly resembles more of morphometrics of wild type. Cardiac inflammation was also common finding in our study, although causes remain unclear and need further investigation. Most of the deviations found in this study were not related to farming site or origin of fish, which indicates that altered heart shape is universal challenge in commercial aquaculture. High prevalence of deviations, asymmetry, inflammation and atherosclerosis may indicate fish being in higher risk of mortality in future, especially if heat waves and handling of fish mid season becomes frequent.

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EFFECTS OF DIETARY LYSOPHOSPHOLIPIDS AND BUTYRATE SUPPLEMENTATION ON GROWTH, FEED UTILIZATION, FILLET FATTY ACIDS, INTESTINAL ENZYMES, INNATE IMMUNITY AND LIVER AND GUT HISTOLOGY IN EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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Lysophospholipids (LPLs) and butyrate, when added in fish diets, are known to have positive effects such as better growth rates, feed utilization, lipid absorption, antimicrobial properties and improvement of the intestine morphology and immune function, in animal nutrition. This study investigated the potential role of the commercial product Product A, which contains LPLs, glyceryl mono-oleate, synthetic emulsifier and a butyrate source to formulate high and low-fat functional diets and characterise their effects on growth performance, feed utilization, intestinal enzymes, gut and liver morphology and immune parameters of European Sea Bass (*Dicentrarchus labrax*). Initial body weight of experimental fish was 18.0 ± 1.9 g and trial duration was 13 weeks. The experimental diets were formulated as follows; Diet 19/0: Positive control high-fat (19%) commercial formula, Diet 19/0.05: Diet 19/0 with the addition of 0.05% Product A, Diet 16/0: Low-fat (16%) control, Diet 16/0.025 and Diet 16/0.05: Diet 16/0 supplemented with 0.025% and 0.05% Product A, respectively. The dietary addition of Product A enhanced fish growth parameters. LPLs supplementation showed a positive effect on high fat diets and no effect in low-fat diets in the fillet fatty acid profiles. Intestinal lipase activity values were improved in all treatments with the inclusion of Product A and the antioxidant activity was slightly enhanced, without statistically significant differences between the treatments. The effects of reducing dietary fat on the fish innate immunity did not showed significant differences with the addition of Product A. Positive effects of the functional low and high-fat diets were also evidenced in the liver histology analysis with significant differences at inclusion levels of 0.05%. In conclusion, these findings show that incorporating Product A at 0.025% allows for a 3% reduction in dietary fat content without negative impacts on growth, feed efficiency, or the health status of juvenile European sea bass within the tested conditions.

Table 1 Scoring system to evaluate histomorphology of liver tissue.

Tanks	Diet 19/0			Diet 19/0.05			Diet 16/0			Diet 16/0.025			Diet 16/0.05		
	A3	A12	B1	A1	A5	B3	A2	A8	B2	A6	A7	A10	A4	A9	A11
	2	3	2	2	2	1	2	2	2	2	2	1	2	1	1
	2	3	2	1	2	1	1	2	2	2	1	1	2	1	1
	2	2	3	2	1	2	2	3	3	2	2	1	1	1	2
Avg./tank	2	2.7	2.3	1.7	1.7	1.3	1.7	2.3	2.3	2	1.7	1	1.7	1	1.3
Avg./diet			2.3c			1.6ab			2.1bc			1.6ab			1.3a

*1 normal morphology = Lightly granular, small and distinct nuclei, 2 moderate alterations = Nuclei with abundant dark granules and cytoplasm homogeneous, vacuolized only to a very limited degree, 3 severe alterations = pyknotic nuclei, large lipidic vacuole within the cytoplasm, displacement of the nucleus. Data are presented as mean scores $\pm$ standard deviation of the means. Different letters in the same column denote statistically significant differences ($P < 0.05$). The data are the means of 3 replicates of 3 fish in each group ($n = 9$).

FUNCTIONAL PROTEIN FROM SILKWORM ENABLES FISHMEAL REDUCTION WITHOUT COMPROMISING ATLANTIC SALMON (*Salmo salar*) PERFORMANCE

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Silkworm pupae meal, a long-standing co-product of sericulture, is increasingly recognized as a valuable insect-derived protein source for aquafeeds. Its favorable amino acid composition, lipid profile, and generally stable nutrient characteristics make it suitable for reducing dependence on traditional marine proteins. However, protein digestibility remains lower. In this study, a process to enhance the protein and digestibility of silkworm pupae was developed and evaluated for its effectiveness for inclusion in aquafeed as a source of protein replacing animal protein sources such as fish meal. Being derived from a waste by-product, this provides for a sustainable source of protein for aquaculture reducing FIFO ratios.

A controlled 4-week feeding study was conducted with juvenile Atlantic salmon (*Salmo salar*) reared in standardized freshwater systems. Diets were produced using a twin-screw extruder with pre-conditioning: a control diet containing 20% fishmeal, and a test diet in which fishmeal was reduced to 15% by incorporating 5% silkworm protein, equivalent to a 25% replacement within the fishmeal fraction. Fish were monitored daily to ensure stable environmental and health conditions throughout the study.

Process development has substantially improved the quality of silkworm protein, enhancing the protein content by 7.8%, digestibility by 10.6%, and reducing the fiber content by 0.7%. Across the 28-day feeding period, salmon offered the silkworm protein diet showed growth performance equivalent to the control group. Final body weight, specific growth rate and eFCR did not differ significantly between the groups ($p > 0.05$). The proximate composition of the silkworm protein diet remained well balanced, with crude protein around 53.3% and lipid near 18.8%.

These results demonstrate that silkworm protein enables a meaningful reduction in fish meal usage without compromising growth. The findings also emphasize the potential of silkworm protein within circular and resource-efficient feed strategies by converting sericulture byproducts into nutritionally relevant aquafeed ingredients.

Further studies over longer production periods and under health-challenge conditions are recommended to evaluate long-term impacts on growth, and potential health benefits.

A FUNCTIONAL SHRIMP PROTEIN HYDROLYSATE FOR LOW FISHMEAL DIET IN ATLANTIC SALMON (*Salmo salar*)

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The rapid expansion of global aquaculture has intensified demand for high quality dietary protein sources, while fishmeal supply has remained largely static for decades, leading to sustainability concerns, market volatility, and pressure on wild fisheries. Reducing fishmeal inclusion in aquafeeds is therefore a strategic priority, but such reductions often compromise feed palatability, nutrient digestibility, and growth performance. Functional protein ingredients derived from responsibly sourced byproducts represent a promising approach to maintaining animal performance while decreasing reliance on finite marine resources. Shrimp protein hydrolysate (SPH), produced from shrimp processing coproducts, has emerged as a potential solution due to its high digestibility, balanced peptide profile, and strong feeding-stimulant properties.

This study evaluated the efficacy of SPH in supporting growth performance and feed utilization in Atlantic salmon (*Salmo salar*) fed low-fishmeal diets. A 56-day controlled feeding trial was conducted using three diets: a high fishmeal control containing 10% fishmeal (positive control); a reduced fishmeal control containing 5% fishmeal supplemented with feather meal hydrolysate; and a reduced fishmeal diet containing 5% fishmeal supplemented with 2.6% SPH. Each dietary treatment was fed to triplicate tanks, with 40 fish per tank, to ensure statistical robustness. Shrimp protein hydrolysate was produced through a fully engineered enzymatic hydrolysis process under optimized conditions, resulting in a high digestibility (>95%) ingredient that rich in short peptides and free amino acids.

At the end of the trial, salmon fed the SPH-supplemented diet showed growth performance and feed efficiency comparable to those fed the high-fishmeal control, despite a 50% reduction in fishmeal inclusion. Final weight gain in the SPH group reached 321 g, closely matching the 335 g observed in the positive control. In contrast, fish fed the reduced fishmeal control without SPH achieved only 291 g weight gain, representing a 10.4% reduction relative to the SPH treatment. Specific growth rate (SGR) followed a similar pattern, with the SPH group (1.32% day⁻¹) performing on par with the high-fishmeal control (1.36% day⁻¹) and significantly outperforming the reduced-fishmeal control (1.22% day⁻¹). Feed conversion ratio (FCR) further confirmed improved feed utilization in the SPH group, which showed no statistical difference from the high-fishmeal control, while the reduced fishmeal control exhibited a significantly higher FCR.

In conclusion, the use of SPH enables a 50% reduction in dietary fishmeal without compromising performance, while simultaneously supporting circular economy principles through the upcycling of shrimp processing byproducts. These findings highlight SPH as a scientifically validated and industry-relevant ingredient for the development of sustainable, high-performance aquafeeds.

SITE SELECTION FOR OPEN OCEAN AQUACULTURE OF LOW-TROPHIC SPECIES

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Offshore aquaculture is increasingly promoted as a solution to rising seafood demand and growing spatial and ecological constraints in coastal waters. Extractive low-trophic species such as bivalves and seaweeds are of particular interest because they can contribute to food production while providing ecosystem services. Yet, sustainable offshore development depends on ecologically robust site selection informed by spatially explicit environmental data.

This study systematically analyses the peer-reviewed literature on site selection for extractive species aquaculture in open-ocean environments, with a focus on how ecological carrying capacity (ECC) is incorporated. Following PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines, studies published between 2000 and 2025 were identified through searches in Scopus and Web of Science. Fourteen studies met the inclusion criteria and were analysed in terms of species focus, geographic distribution, methodological approach, and degree of ECC integration.

Results show that GIS-based multi-criteria evaluation is the dominant method used for offshore site selection, typically combining environmental, technical, and spatial-use constraints. However, most studies stop at suitability mapping and do not explicitly address ECC. Only a limited number of studies apply mechanistic approaches, such as Dynamic Energy Budget models, Farm Aquaculture Resource Management model or coupled hydrodynamic-biogeochemical models to account for ecosystem feedbacks and sustainable production limits.

The review identifies a clear need to move from static suitability assessments towards integrated, ecosystem-informed frameworks that include adaptive thresholds and mechanistic modelling. Embedding ECC into offshore site selection will be critical for sustainable aquaculture expansion and effective marine spatial planning, particularly in the context of the growing interest in multi-use systems involving offshore wind farms.

Acknowledgment

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DRIVERS OF SALMON LOUSE CONSUMPTION IN LUMPFISH (*Cyclopterus lumpus*): EFFECTS OF PREY AVAILABILITY, FISH TRAITS AND DEPLOYMENT CONDITIONS

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Lumpfish (*Cyclopterus lumpus*) are used as a non-pharmacological method to control salmon lice (*Lepeophtheirus salmonis*) in Atlantic salmon (*Salmo salar*) aquaculture. However, their effect as cleaner fish varies substantially. This study examines how alternative prey availability, individual characteristics and deployment strategy influence salmon lice consumption in lumpfish, aiming to improve decision-making and effectiveness of lumpfish-based lice control in salmon aquaculture. Stomach-content data from 28,677 lumpfish sampled across multiple sites and years in the Faroe Islands (2015-2025) were analysed. The dataset included fish size and body condition, presence of alternative prey in stomachs, and cage-level salmon lice abundance on cohabiting salmon. Most lumpfish stomachs (>96%) contained no salmon lice, indicating low treatment engagement under typical farming conditions. Due to the high proportion of zeros and large variation in salmon lice counts (0–290), a probability-based modelling approach was used to describe both the likelihood and the intensity of salmon lice consumption. The probability of salmon lice consumption increased with salmon louse abundance on cohabiting salmon and was strongly reduced when jellyfish were present in stomachs. Consumption intensity showed non-linear relationships with salmon louse abundance, lumpfish size and body condition, peaking at small sizes and optimal condition. These results indicate that salmon lice consumption is opportunistic and strongly influenced by prey availability and deployment conditions. Avoiding deployment during periods dominated by jellyfish blooms, accounting for salmon lice levels at deployment and maintaining good body condition through appropriate feeding and husbandry may improve the effectiveness and targeted use of lumpfish as a biological control agent.

HOW COOL IS PIKEPERCH (*Sander lucioperca*)?

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Introduction

Percids are coolwater species, and while thermophilic in terms of growth, they require a cold period for reproduction (Embrke et al., 2025). While pikeperch (*Sander lucioperca*) requires cooling for maturation of its gonads, it is yet speculative what the lowest temperature is required for its successful reproduction (Hokanson et al., 1977). Thus far, there have been two studies evaluating this topic (Hermelink et al., 2017; Milla et al., 2021). Although both studies showed that a relatively high temperature of 12 °C was appropriate for full gonadogenesis, fish reproduction was not reported, so the gamete quality was not assessed. Accordingly, to the best of our knowledge, the study that reported reproduction with the warmest wintering temperature was at about 9 °C (Ljuboratić et al., 2020). On the other hand, the study that reported the lowest temperature for the successful reproduction was at 10 °C (Ljuboratić et al., 2021). Building upon the previous knowledge, yet aiming to reach a feasible spawning induction protocol, the present study evaluated the egg quality in two batches of females with different reproductive experience when the chilling phase was performed at 10 °C.

Materials and methods

In total, 20 females were used for the evaluation of the egg quality. Prior to induction in fully controlled conditions, fish were raised outdoors in in-pond raceways for three years, when the spawning cycle shift was performed (Ljuboratić et al., 2025). Ten females were seven years old and had already been artificially reproduced twice since the cycle shift (OLD), while the other ten were five years old and had not been artificially reproduced thus far, and had just been submitted to the reproductive cycle shift for the study (YOUNG). Mimicked summer was performed at a stable temperature of 21 °C with constant daylight reduction of 2 min/day from 15.5h to 12h. Autumn was mimicked with gradual cooling from 21 °C to 10 °C at a rate of 1°C weekly, while the daylight period was further reduced to a minimal 8.8h with stable dynamics of 2 min daily. Further on, the stable 10 °C was kept, while the daylight increased with a dynamic of 3 min/day. Upon one (EARLY) or two months (LATE) of this stable chilling phase, ten fish (five from each class) were hormonally stimulated and reproduced by the stable thermal protocol described by Ljuboratić et al. (2021). In short, from hormonal stimulation until the GVBD, the temperature was kept at 10 °C, while upon reaching the germinal vesicle breakdown, the fish was moved to 12 °C until ovulation, which took place within 24 h.

Results

Latency time was affected by both induction moment and the age of the breeders, being on average 2 days and 4.5 days shorter in YOUNG, for EARLY and LATE reproduction batches, respectively. Embryo survival rate was relatively high in both batches, with a mean about 10 % higher in the EARLY batch and ranging from 31.4 % to 78.8 % and from 49.3 % to 87.0%, in the EARLY and LATE batches. Embryo survival was not affected by either of the two assessed factors. Oil globule fragmentation was affected by the induction duration only, being higher in the LATE batch (24.2 ± 37.5 % vs. 2.7 ± 4.8 %).

Discussion

The present results show that pikeperch's ability to produce high-quality eggs upon hormonal stimulation of ovulation, even with the chilling phase being at 10 °C, yet offering a one-month period of propagation similar to earlier reports at lower temperatures (Ljuboratić et al., 2020). Based on the assessed egg quality features, it appears that even after the repeated cycles in the shifted reproduction regime, females are still capable of yielding high reproductive performance, ensuring the reliability of the protocol. The presented spawning induction schedule, following the reproduction cycle shift, presents a new step forward towards the economic feasibility of pikeperch off-season egg supply.

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MULTI-METHOD KEYSTONE SPECIES IDENTIFICATION REVEALS DISTRIBUTED ECOLOGICAL RESILIENCE IN AQUAPONIC MICROBIAL COMMUNITIES

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Introduction

Aquaponics integrates recirculating aquaculture with soilless plant production in a shared water circuit, relying on microbial communities to mediate nutrient cycling underpinning system stability. Whether individual keystone species - taxa whose removal would disproportionately restructure community composition relative to their abundance - exist in aquaponic systems remains unknown (Garza et al., 2026; Rottjers & Faust, 2023). Identifying such taxa, or confirming their absence, would clarify the ecological architecture of these systems and inform microbiome-based monitoring and management strategies. Prior work has combined presence-impact statistics, co-occurrence network topology, and machine-learning approaches in other natural aquatic ecosystems, but not in recirculating aquaculture or aquaponics.

Methods

Eighty-eight 16S rRNA V4 amplicon libraries were collected from three compartments (clarifier, fish tank, grow bed) across six sampling dates (May–November 2024) at a research-scale facility (Auburn University, USA) operated under coupled and decoupled hydraulic configurations. After DADA2 processing with cutadapt primer trimming-which reduced chimera rates from 85.7% to acceptable levels-825 ASVs were retained after prevalence filtering ($\geq 20\%$ of samples; 61,787 total ASVs). Three independent keystone screens were applied: (i) leave-one-out PERMANOVA presence-impact analysis, in which each focal taxon was removed before Bray–Curtis distance calculation to avoid circularity, with compartment and hydraulic coupling as covariates and false discovery rate (FDR) correction (150 candidate taxa tested); (ii) Spearman co-occurrence network hub identification among the 50 most CLR-variable taxa ($n = 60$ samples; $|q| > 0.6$, $\text{padj} < 0.001$); and (iii) LASSO regression predicting Bray–Curtis dissimilarity between consecutive samples at each location.

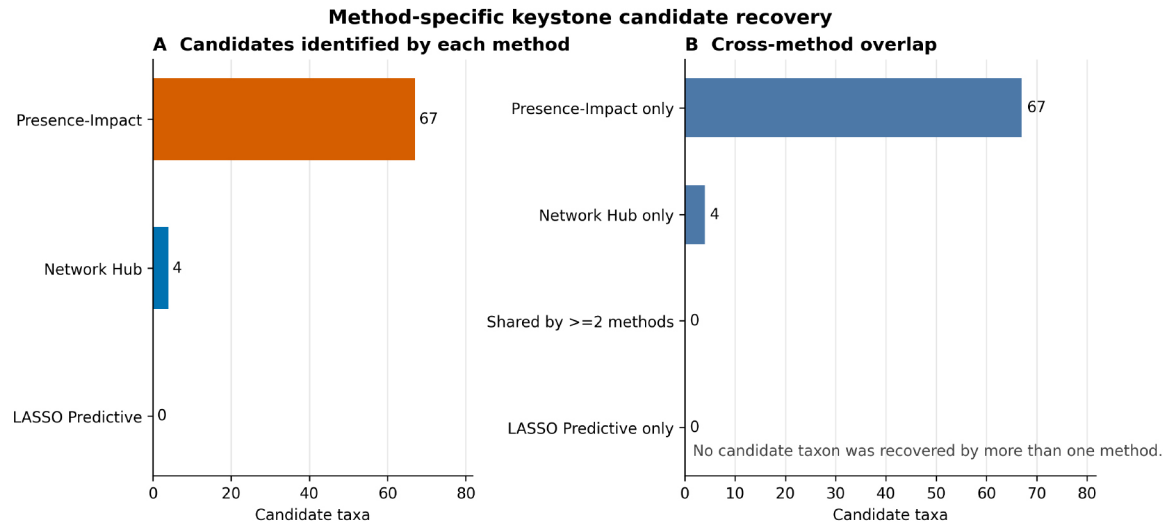


Figure 1. Non-overlapping keystone candidates across three detection methods. UpSet plot showing set membership of the 71 unique candidates recovered from presence-impact PERMANOVA (67 taxa), co-occurrence network hubs (4 taxa), and LASSO temporal prediction (0 taxa). No taxon was supported by more than one method.

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Results

A distributed ecological resilience framework, rather than dependence on a few keystone taxa, appears to best describe the aquaponic environment. Compartment was the dominant driver of community structure (PERMANOVA $R^2 = 0.256$), with smaller contributions from hydraulic coupling ($R^2 = 0.069$), plant growing season ($R^2 = 0.055$), and illumination regime ($R^2 = 0.031$). Grow bed samples showed the highest alpha diversity; fish tank samples showed the lowest. Leave-one-out PERMANOVA identified 67 taxa at $FDR < 0.05$ and $R^2 > 0.02$. *Cetobacterium* sp. (Fusobacteriota) ranked highest, with three ASVs each explaining 8.2–11.0% of community variance—a signal driven by its restriction to fish tank samples. Other high-impact taxa included Dysgonomonadaceae ($R^2 = 0.067$), *Thermomonas* sp. ($R^2 = 0.054$), and *Ferruginibacter* sp. ($R^2 = 0.045$), spanning four phyla. The co-occurrence network yielded 50 nodes and 335 edges (density = 0.27); four biofilm-associated hub taxa were identified across Proteobacteria, Planctomycetota, and Armatimonadota. LASSO retained zero individual taxa as predictors of temporal turnover. Critically, no taxon appeared in more than one detection method: presence-impact candidates and network hubs were entirely non-overlapping (Figure 1).

Discussion

The absence of cross-method consensus is the key finding: aquaponic systems do not appear to harbor classical keystone taxa as in natural aquatic environments. The pattern is consistent with distributed ecological resilience, where community-structuring function is spread across many taxa rather than concentrated in a few irreplaceable ones (Allison & Martiny, 2008). One genus, *Cetobacterium*, may serve as a compositional indicator in fish tanks, but not as a system-wide keystone; its strong presence-impact signal appears to result from compartment specificity. Network hub taxa reflect co-response to shared environmental drivers rather than direct metabolic control, as shown by the inability of graphical LASSO to recover a sparse network even at maximum regularization.

Operationally, these findings support compartment-level community monitoring over surveillance of universal keystone targets.

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CO-INJECTION OF PHARMAQ MICRO-DOSE EMULSION VACCINES

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The adoption of waterin oil (W/O) emulsion vaccines has been central to disease prevention strategies in modern aquaculture. To further improve fish welfare, growth performance, and operational efficiency, PHARMAQ introduced the microdose vaccination concept, reducing the injectable volume from 0.1 mL to 0.05 mL while maintaining high efficacy. The development and implementation of microdose vaccines place stringent requirements on antigen production, formulation robustness, and vaccination accuracy, particularly when multiple vaccines are administered simultaneously by coinjection.

Coinjection, defined as the simultaneous administration of two or more vaccines through a single needle, has become common practice in salmon aquaculture and allows high flexibility in vaccine combinations while limiting the fish to a single puncture. However, this approach introduces additional technical challenges, including vaccine compatibility and the need for highly accurate and precise delivery of each individual dose component.

This contribution provides an overview of formulation aspects of PHARMAQ microdose W/O emulsion vaccines, administration principles, and coinjection strategies used in commercial salmon vaccination. Particular emphasis is placed on the evaluation of vaccination equipment performance. Accuracy and precision of dosing were assessed using established gravimetric methods for single channel injection. For coinjection, where conventional weighing cannot discriminate between individual vaccine volumes, a fluorescence based analytical method was developed. Model vaccines with flow properties matching commercial microdose emulsions were formulated, enabling quantification of the individual contribution of each vaccine within a single coinjected dose.

Using this approach, commercially available vaccination equipment from multiple manufacturers was evaluated. The results demonstrate high accuracy and precision for both single injection and coinjection, with individual vaccine volumes consistently delivered within narrow acceptance limits. These findings confirm that, when combined with robust formulation development and validated administration systems, microdose coinjection can be implemented reliably under commercial conditions.

Overall, this work highlights the importance of integrating vaccine formulation, dosing technology, and analytical methodology to ensure consistent performance of microdose emulsion vaccines in modern aquaculture vaccination practices.

THE INVASIVE BLUE CRAB *Callinectes sapidus* FROM THE NORTHERN ADRIATIC SEA: FEEDING BEHAVIOUR AND FECUNDITY DATA

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Introduction

The blue crab *Callinectes sapidus* is currently one of the most problematic invasive species in the Mediterranean Sea [1-3], particularly along the Italian coasts. Since its first record in the northern Adriatic in 1949, the species has progressively expanded, colonising most Italian coastal and lagoon systems. Its invasion success is driven by a combination of biological and ecological factors, including high tolerance to variations in temperature and salinity, an extremely flexible and opportunistic diet, and a remarkable reproductive capacity. From an ecological perspective, the blue crab is an aggressive omnivorous predator capable of feeding on a wide range of organisms, including fish, crustaceans, and especially bivalve mollusks. This trait makes it particularly impactful in the lagoon systems of the northern Adriatic, where clam and mussel aquaculture are widespread [8]. Predation on these organisms represents one of the main economic threats to the sector. Alongside its trophic role, reproduction is another key aspect: females can produce millions of eggs per reproductive cycle and spawn multiple times, contributing to rapid population growth [7]. In addition, environmental factors such as increasing temperatures may further enhance the spread of the species [3-6]. In light of these issues, the present study aims to integrate the analysis of feeding behaviour and reproductive potential of the blue crab in order to better understand the mechanisms underlying its invasive success and to provide useful information for species management.

Materials and Methods

Blue crab specimens used in this study were collected in the Bevano River Delta (northern Adriatic Sea) and a fishing house in Cesenatico, during the summer of 2025. After capture, the animals were transported to the laboratory and acclimatised in a controlled recirculating aquaculture system (RAS) that ensured stable environmental conditions in terms of temperature, oxygen, salinity, and pH. Biometric measurements were performed to classify individuals according to sex and size, and the animals were then divided into groups and subjected to different experiments. Regarding feeding behaviour, three dietary treatments were tested: a monospecific diet of clams (*Ruditapes philippinarum*), a monospecific diet of mussels (*Mytilus galloprovincialis*), and a mixed diet. Food was provided daily, and both the number of consumed prey items and the ingested biomass were recorded. In parallel, prey handling and consumption times were analysed, recording the time required by crabs to open and consume each prey type. A further experiment evaluated the effect of temperature on feeding activity by progressively varying water temperature and recording food consumption across eight temperature levels (13–34 °C). Finally, reproductive performance was assessed using ovigerous females. Egg masses were weighed and subsampled to estimate total egg number, egg diameter, and developmental stage, as well as to evaluate the relationship between female body size and absolute fecundity.

Results

The results show that *C. sapidus* is able to consume both tested bivalve species without a clear numerical preference. Under mixed diet conditions, all size and sex groups showed very similar numbers of clams and mussels consumed. However, a clear difference emerged when edible biomass was considered. The mean edible tissue per prey item was 1.74 ± 0.52 g for clams and significantly higher at 4.50 ± 1.01 g for mussels. In the mixed diet trial, despite similar prey counts, mussels provided a significantly greater total edible biomass (47.75 ± 7.98 g vs 25.21 ± 2.98 g; $p < 0.05$). Regarding handling and consumption times, differences between prey types were limited. Mean valve-opening time ranged from 1 min 12 s to 1 min 16 s for clams and from 50 s to 1 min 17 s for mussels. Tissue consumption time was slightly longer for mussels than for clams. Total processing time ranged from approximately 2 min 50 s to 3 min 25 s across prey types, with no statistically significant differences detected ($p > 0.05$). Temperature had a clearly non-linear effect on food intake. Consumption increased progressively from 13 °C, reaching a peak at 25–28 °C, and then declined at 31–34 °C. At 13 °C, consumption was reduced by approximately 84% compared to the optimal peak, indicating a strong thermal limitation of feeding activity. Reproductive analysis revealed high fecundity, with absolute egg number ranging from approximately

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958,000 to 2,736,000 eggs per female and a mean of $1,632,558 \pm 530,098$ eggs per individual. Egg mass represented on average $20.45 \pm 3.1\%$ of total body weight. A strong positive correlation was detected between carapace width and absolute fecundity ($p < 0.001$), indicating that larger females produce significantly more eggs.

Discussion

The results confirm the role of *C. sapidus* as an extremely efficient and adaptable predator. Its ability to exploit different prey types and optimise the balance between prey number and ingested biomass represents a significant competitive advantage, particularly in resource-rich environments such as the northern Adriatic lagoons [4,5]. Although clams are easily accessible on the seabed, mussels provide higher energetic returns per prey item, which may influence prey selection under natural conditions [4]. However, the actual impact on aquaculture also depends on farming system structure, prey accessibility, and habitat characteristics, and the present results — obtained under controlled laboratory conditions without sediment — should be interpreted as estimates of potential consumption rather than direct predictors of field impact [5]. Temperature is confirmed as a key factor regulating feeding activity [6]. The unimodal thermal response, with peak consumption at 25–28 °C and reduced intake at both thermal extremes, is consistent with the physiology of ectothermic predators. The marked reduction at 13 °C is consistent with cold-induced inactivity, while the decline above 28 °C suggests entry into a supra-optimal thermal range. From an applied perspective, climate warming may extend the period of peak feeding activity, potentially increasing predation pressure on shellfish aquaculture [3,8]. Finally, high fecundity represents one of the most critical aspects for the management of this species [7]. The production of over 1.6 million eggs per female per brood, combined with multiple reproductive cycles, ensures rapid population expansion. The positive relationship between body size and fecundity underlines the key role of larger individuals in sustaining population growth. Taken together, the high trophic efficiency and exceptional reproductive capacity of *C. sapidus*, combined with its broad environmental tolerance, make it a particularly challenging invasive species and highlight the need for integrated and evidence-based management strategies.

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VARIANT PRIORITIZATION BASED ON FUNCTIONAL AND EVOLUTIONARY SIGNIFICANCE SCORES IMPROVES GENOMIC PREDICTION OF A COMPLEX TRAIT IN THE EUROPEAN SEA BASS (*Dicentrarchus labrax*)

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Introduction

In aquaculture species, traditional selective breeding for complex traits based on estimated breeding values (EBVs) is often not feasible due to the costs and challenges associated with phenotyping. While genomic selection (GS) leveraging high-density SNP genotype data offers a more efficient approach to improve complex traits, whole-genome sequencing (WGS) has frequently yielded negligible accuracy gains in genomic prediction (Perez-Enciso *et al.*, 2017). This is mainly caused by redundant, non-informative variants, generating noise that masks real informative variants. To “rescue” the WGS potential in GS, this study investigates, for the first time in an aquaculture species, a variant prioritization strategy based on the Functional-And-Evolutionary Trait Heritability (FAETH) score (Xiang *et al.*, 2019). This approach was applied to the European sea bass (*Dicentrarchus labrax*) to refine genomic prediction for a complex trait, disease resistance, with viral nervous necrosis (VNN) resistance as a representative case study.

Materials and Methods

990 sea bass were phenotyped for body length (mm) and binary VNN mortality following a 29-day experimental challenge. Experimental fish were genotyped using the Med-Fish SNP array (27,740 SNPs; Peñaloza *et al.*, 2021) and imputed to WGS (6,072,853 SNPs) using a reference panel of sequenced parents (25 sires and 25 dams) and 40 experimental fish. Six functional and evolutionary categories, namely ATAC/ChIP-seq data (from the AQUA-FAANG project; <https://www.aqua-faang.eu/>), Ensembl regulatory features, Variant Effect Predictor consequences, genomic islands of differentiation between the Atlantic and Mediterranean bass populations, linkage disequilibrium (LD) and minor allele frequency score quartiles, were used to partition the WGS dataset into 30 variant sets. Each set was converted into a genomic relationship matrix to estimate the SNP-based heritability (h^2) using GCTA (Yang *et al.*, 2011). The estimate was divided by the number of variants in each set, yielding a mean per-variant heritability (h^2_{var}). The FAETH score was calculated as the mean of h^2_{var} across both traits and all six categories. Variant subsets including SNPs with the highest FAETH scores (top 1%, 5%, 10%, 25%, and 50%) were used in prediction of genomic estimated breeding values (GEBVs) for VNN resistance and compared with the 25% lowest-ranking SNPs. GEBVs were obtained using Bayesian threshold models (GIBBSf90+/BLUPf90+; Misztal *et al.*, 2018). Eight scenarios were considered: four LD-pruning filters (no filtering, and thresholds of 0.99, 0.95 and 0.80), each combined with either the inclusion or exclusion of a major QTL for VNN resistance on chromosome 3 (Mukiibi *et al.*, 2025). A 2-fold cross-validation, minimising genetic relatedness between training and testing sets, was employed, and prediction accuracy was calculated as

$$r_{\text{adj}} = \sqrt{\text{corr}(\text{EBV}, \text{GEBV})^2 * \left(1 - \left(\frac{\text{PEV}}{\sigma_a^2}\right)\right)}, \text{ where } \text{corr}(\text{EBV}, \text{GEBV}) \text{ is the Pearson correlation between}$$

EBV and GEBV, PEV is the prediction error variance and σ_a^2 is the additive genetic variance of the trait. Genomic predictions were replicated 100 times for each variant subset, each time randomly sampling 2,000 SNPs from the target subset.

Results

For VNN mortality and body length, estimates ranged from 0.35 to 0.42 and from 0.40 to 0.56, respectively. Regarding , nine of the top ten sets belonged to the ATAC/ChIP seq functional category. Prediction accuracies consistently improved when using high FAETH-ranked variant subsets across all eight scenarios. In the “No LD pruning” scenario, accuracy increased by 1.18% (top 50%) to 6.90% (top 1%) relative to the subset consisting of 25% low-ranking SNPs. In the “LD pruning 0.99” and “LD pruning 0.95” scenarios, similar predictive gains were observed, ranging from +3.30% (top 25%) to +5.41% (top 10%), and from +3.94% (top 25%) to +6.93% (top 1%), respectively. Under the “LD pruning 0.80” scenario, gains were even larger, ranging from +4.05% (top 25%) to +8.10% (top 10%). Excluding chromosome 3 reduced overall prediction accuracy by approximately 3-4%; however, the trend of increased accuracy remained consistent.

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Discussion

This study represents the first attempt in an aquaculture species to apply a variant prioritization strategy that leverages WGS data for genomic prediction of a complex trait using the FAETH score classification. Genomic prediction accuracies for VNN resistance improved consistently for high-ranking subsets, across all eight scenarios. Notably, we observed an increasing trend in accuracy as data was restricted to the highest-ranking variants and under more stringent LD pruning scenarios. We hypothesize that subsets consisting of top-ranking variants are less susceptible to redundancy-induced noise and exhibit a greater capacity to preserve performance compared to the low-ranking variant subset. These findings suggest that the FAETH score might be a reliable strategy to enhance genomic prediction accuracy for complex traits in aquaculture species.

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INCREASING GENETIC PROGRESS USING CANDIDATES GROWN UNDER PRODUCTION LIKE CONDITIONS: USE OF GENTIC SIMULTATIONS TO INFORM BREEDING PROGRAMME DESIGN

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Genetic selection programmes must maintain a balance between selecting for performance under realistic production conditions and protecting the integrity of the breeding population. Salmonid breeding programmes often maintain nucleus candidates in biosecure freshwater facilities to minimise the risk of a catastrophic loss of genetics that may occur following mass mortality caused by pathogen outbreaks or environmental stresses. Selection for commercial traits can be applied by testing full-siblings of the selection candidates in sea cages, but this design results in reduced breeding value accuracy in the selection candidates causing reduced potential for genetic gain. Whilst genomic selection can, to a large extent, address this shortfall, such approaches are not always feasible in species with limited genomic resources, or in integrated programmes that supply relatively small production volumes, where the cost of high-density genotyping can be prohibitive.

Maintaining some, or all, of the selection candidates in production-like conditions is a viable strategy for maintaining strong rates of genetic progress but comes with substantial biosecurity risks and operational complexity. Deciding on an appropriate breeding programme design requires a detailed understanding of the likely impacts of these choices.

Genetic simulations provide a valuable decision-making framework to test the potential outcomes from contrasting breeding programme designs. We present results from a simulation study examining factors affecting the commercial return when varying proportions of candidates are grown in the production environment and compare these to conventional pedigree-based selection and genomic selection methods. Our results demonstrate that the use of candidates can provide high rates of genetic gain compared to traditional approaches, but optimal design is dependent on the production volume supported.

We illustrate these results with an example from a chinook salmon breeding programme where all candidate are grown in salt-water and spawned directly from sea-cages using a combination of phenotypic pre-selection and genetically informed mating plans. Under this management, this programme has achieved a consistent reduction in time to harvest weight, demonstrating the potential effectiveness of this approach.

FEEDING RAINBOW TROUT WITH *Hermetia illucens* AND *Spirulina* MEALS: EFFECTS ON GROWTH PERFORMANCE, NUTRIENT DIGESTIBILITY, AND DIGESTIVE SYSTEM HEALTH

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Introduction

The last decade, there has been a growing need for new aquafeeds ingredients that meet nutritional requirements while reducing environmental impact (Gasco et al., 2023). Two promising alternatives to fishmeal are insect and microalgae meals. Insect-based diets are rich in proteins and lipids (Gasco et al., 2023), while microalgae provide key nutrients such as n-3 highly unsaturated fatty acids, essential for proper fish growth (Conceição et al., 2010). Several studies have shown that these alternative protein sources can also improve fish stress response and disease resistance (Yadav et al., 2020). This study evaluated the use of *Hermetia illucens* (HI) and *Spirulina* (SP) meals –individually and in combination– as feed ingredients for *Oncorhynchus mykiss* (rainbow trout), with the aim of identifying optimal inclusion levels to enhance growth performance, nutrient digestibility, and the health of the digestive system.

Materials and Methods

Seven isonitrogenous, isolipidic, and isoenergetic diets were formulated: a control diet (CTRL) containing 20% of fish meal, and six diets where the fish meal was 100% replaced with *Hermetia illucens* meal (HI100), 100% *Spirulina* meal (SP100), 75% HI and 25% SP (MIX1), 66.6% HI and 33.3% SP (MIX2), 33.3% HI and 66.6% SP (MIX3), and 25% HI and 75% SP (MIX4). To evaluate nutrient digestibility, 1% of indigestible marker was added to all the diets. For the growth trial, a total of 441 fish (initial body weight of 200.5 ± 2.6 g) were randomly allotted to 21 400L-tanks (3 replicates/diet, 21 fish/tank). Feed was hand-distributed twice daily, seven days a week. Daily rations were set as a % of the tank biomass and adjusted every 14 days based on fish growth and water temperature. The trial lasted 88 days. At the end, growth performance indices were calculated, and 10 fish per tank were slaughtered to determine the somatic indices (n=6) and the histomorphological changes of the intestine, liver, and spleen (n=4). The Apparent Digestibility Coefficients (ADCs) were measured by performing a digestibility trial, where 165 fish (initial body weight 239.6 ± 0.09 g) were randomly allotted to 11 250L-tanks. The experiment was conducted in two consecutive runs, with diet reallocation ensuring three replicates per diet across different tanks. A 7-day adaptation period was applied prior to each fecal collection phase. The fish were fed *ad libitum* and the feces were collected twice daily from each tank for four weeks using a continuous automatic device (Choubert et al., 1982).

Results

Growth performance and somatic indices were not affected by diet ($p > 0.05$). The histomorphology evaluation of the anterior intestine showed that the fish fed with SP100 and MIX4 had smaller villi (length and surface), lower number of goblet cells/100µm, and thinner mucosal, submucosal, and muscularis layers when compared to the CTRL, HI100, and MIX1 diets ($p < 0.05$). However, there was no evidence of the effect of the diets on the pathology of the digestive organs ($p > 0.05$). Finally, the ADC of dry matter was not affected by the diet ($p > 0.05$), while the fish fed with the SP100 diet was less able to digest crude protein compared to the CTRL diet ($p < 0.05$) and both the diets with the highest inclusion levels of *Spirulina* (SP100 and MIX4) affected negatively the digestion of the ether extract compare to the CTRL, HI100, and MIX1 diets ($p < 0.05$).

Discussion

The present study provides novel insight into the effect of the total replacement of the fish meal with HI and/or SP meals in rainbow trout. The growth performance of the fish was not different between the CTRL and the experimental diets, thus being in agreement with previous studies (Sheikhzadeh et al., 2019a, Biasato et al., 2022). Even though low inclusion levels of *Spirulina* can have a positive effect on the digestive system and digestibility of the fish (Zhang et al., 2024), in the present study, the diets with the highest inclusion percentages of *Spirulina* (SP100 and MIX4) displayed deterioration of the intestine morphology and liver's health, and lower digestion of the protein and the lipids. The results showed that the diets with the highest inclusion levels of *Hermetia illucens* meal (HI100 and MIX1) were the optimal ones in terms of the health of the intestine and the digestion of the fish.

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COLOUR ENHANCEMENT OF *Paracentrotus lividus* ROE USING FEED PRIMING WITH GREEN AND RED SEAWEED

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Introduction

The increasing market demand for sea urchin roe has been fostering research to produce sea urchins with high-value gonads. The distinct golden-orange to yellow colour of sea urchin gonads results from the accumulation of carotenoid pigments provided by a wide variety of seaweeds they feed on in their natural habitat (Robinson et al., 2002). The European sea urchin *Paracentrotus lividus* (Lamarck, 1816) is the most important emergent sea urchin to be produced in Southern Europe, including Portugal. Here, the market demand for sea urchin roe is small. However, the harvesting pressure on *P. lividus* is increasing to meet the growing markets in Spain, Italy, and France (Lourenço et al., 2021). The production of high-quality sea urchin roe using the practical dry feed is a difficult task, mainly due to an imbalance between dietary nutritional requirements, the supplementation with natural carotenoid pigments, and feed production processes. Roe priming using supplementary sea urchin feeding with fresh and dried seaweed appears as a feasible approach to produce high-quality roe. In the present study, we aimed to enhance the roe colour of sea urchin by combining sea urchin feeding with dry feed and a final priming stage of exclusive feeding with seaweeds.

Materials and Methods

An enhancement trial was conducted with two stages: an initial stage to promote the gonad growth, and a final stage to tune the gonad colour. In the gonad growth stage, the sea urchins were fed for 12 weeks with three experimental diets of identical proximate composition formulated with ingredients of algal, animal and vegetable sources. In the priming stage, the sea urchins were fed, for four weeks, with a green macroalgae (*Ulva rigida*) and a red macroalgae (*Porphyra dioica*). The efficiency of each phase was analysed in terms of sea urchin growth performance, gonad proximate composition, including fatty acid and carotenoid pigment profiles, colour and texture quantitative determination and sensory characteristics.

Results and Discussion

The results showed that, after an initial stage of gonad growth as a result of bulk feeding with the dry feeds, the period of exclusive algae feeding improved the colour of gonads with a small but significant reduction in sea urchin gonadosomatic index. The higher colour score observed was followed by significant changes in the carotenoid pigments profile, mainly in echinenone and lutein concentration in the gonads. As in previous studies, the changes observed in gonad colour and carotenoid pigment profile were affected by sea urchin sex. In conclusion, exclusive seaweed feeding can be used as a priming strategy to improve the roe quality of sea urchins produced in aquaculture.

Acknowledgment

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A HOLISTIC APPROACH TO PERFORMANCE ANALYSIS IN TEMPERATURE-CHALLENGED RAINBOW TROUT *Oncorhynchus mykiss* - THE IMPACT OF DIETARY DIFORMATES

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Introduction

Aquaculture is currently the fastest-growing food production sector worldwide and plays an important role in global food security, providing more than 50% of all aquatic products consumed. Within this context, global Rainbow trout production has reached close to 1 Mio t annually and its global market was valued around USD 4.5 billion in 2024. Climate change, and as a result increasing water temperatures with related effects (e.g. hypoxia) are additional stressors to many aquatic species, especially salmonids (Stowbridge et al., 2025). On the other hand, results indicate that growth performance, nutrient digestibility and gut health, can be improved by some organic acids and their salts, particularly formic acid salts in some fish species, also under challenged conditions (Lückstädt, 2012). The beneficial impacts of those dietary acidifiers result mainly from strong antimicrobial effects and digestibility enhancing properties. Usage of acidifiers in aquaculture was pioneered a couple of decades ago and several review papers (Lückstädt, 2008; Fabay et al., 2022; Chen et al., 2024) have confirmed the impact across numerous species. However, data on the effect in trout under challenged conditions were scarce. This formed the impetus for the trial.

Materials and Methods

A trial to test the hypothesis of a beneficial impact of acidifiers during temperature challenge was carried out with all female, diploid Rainbow trout (*Oncorhynchus mykiss*; initial weight 119 g) over a period of 62 days in a recirculating aquaculture system under suboptimal environmental conditions (22°C water temperature) at a commercial research facility in northern Germany. The treatment group included one of the most widely used acidifier in aquaculture globally, based on the patented diformate concept (Formi® NDF, ADDCON, Norway; hereafter abbreviated as NDF), at 0.5%. During the trial the following parameters were monitored: Final weight (g), Specific growth rate SGR (%), Feed Conversion Ratio FCR, Slaughter yield SY (% and g) and Fish Productivity Index FPI. The FPI is calculated based on the following formula: $\text{Weight gain [g]} \times \text{Survival [\%]} / (10 \times \text{FCR})$, thus including the most important parameters in fish production. Furthermore, a slaughter yield based performance index was introduced – the Slaughter Yield Productivity Index SYPI, which is calculated according to the following equation: $\text{SY [\%]} \times \text{Survival [\%]} / (10 \times \text{FCR})$. Additionally, some health-indices (skin health, fin health) were observed.

The data were statistically analysed and a $P < 0.05$ value was considered significant.

Results

During the trial period, fish fed the control diet as well as the NDF-diets tripled in size, indicating high feed palatability and growth, despite the challenge. Overall, both groups showed a high survival rate (>99%). The Skin- and Fin Health Indices showed no severe cases in the NDF-fed trout, a reduction from more than 3% in the control group. Trout fed the test diet showed a trend towards slightly better SGR (+1.1%) and FCR (-1.0%). The FPI was numerically improved by 2.7%, while the slaughter yield (%) improved significantly ($P=0.02$) by 1.7% or by 2.5% ($P=0.002$), when the fish size was additionally taken into account. Finally, the SYPI was determined to be highly significantly improved by 3.9% ($P < 0.0001$) in the trout receiving the dietary NDF.

Discussion

As stated by Farsani et al. (2020) and Chen et al. (2024), the dietary diformate (in those cases potassium diformate) exhibits various effects, including antibacterial activity, growth promotion, an improved feed conversion ratio (FCR) and the regulation of intestinal microbiota in Rainbow trout under optimal conditions. Our data reveal that this holds also true for the culture of Rainbow trout under temperature stress when fed with dietary sodium diformate (NDF). It can be concluded that the diformate based acidifier is able to improve health and performance in Rainbow trout also under challenged conditions.

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ASC'S CLIMATE STRATEGY: BUILDING TRANSPARENCY, MITIGATION, AND RESILIENCE IN AQUACULTURE

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Introduction

Aquaculture has the potential to provide a growing global population with high-quality and low emission proteins, yet its climate impacts and vulnerabilities remain poorly understood and addressed. While the sector has made progress in sustainability, a gap persists between high-level climate commitments and practical, on-the-ground action, particularly in measuring emissions, protecting ecosystems, and building resilience. Aquaculture Stewardship Council (ASC) is addressing these challenges through a comprehensive Climate Strategy, designed to provide clarity, tools, and frameworks for producers, supply chains, and stakeholders.

Methodology

The strategy was developed through a multi-stakeholder process, engaging producers, NGOs, scientists, policymakers, and financial institutions. Workshops, consultations, and targeted research identified key barriers to climate action, including limited emissions data for most species, knowledge gaps, and financial constraints. The resulting strategy is structured around three interconnected pillars, each addressing a critical aspect of climate-smart aquaculture.

Strategy Pillars

Measurement and Reporting: The first pillar focuses on establishing a clear, standardized understanding of the industry's greenhouse gas emissions. ASC has developed a GHG calculator to measure and benchmark emissions by species and production system, providing producers with the data needed to assess their climate footprint and set informed baselines for future action. This foundational work prioritizes transparency and accountability, enabling the sector to move from uncertainty to evidence-based decision-making.

Mitigation through Ecosystem Protection and Restoration: The second pillar advances mitigation by protecting and restoring critical ecosystems. ASC is strengthening our farm and feed standards for habitat conservation, such as mangrove and seagrass protection, as well as terrestrial systems like forests and grasslands. By aligning with global biodiversity goals and collaborating with conservation partners, this pillar ensures that mitigation efforts are both effective and ecologically beneficial.

Adaptation and Resilience: The third pillar addresses the urgent need for practical, actionable resilience. ASC's strategy includes risk assessment frameworks, capacity-building programs, and financial innovations to help producers and supply chain actors prepare for climate-related challenges. Knowledge-sharing platforms will facilitate the exchange of best practices, ensuring that resilience efforts are collaborative, adaptive, and grounded in real-world needs.

Implementation

With the strategy finalized, ASC is now focused on operationalizing the three pillars in collaboration with industry and stakeholders. This includes supporting producers in adopting measurement tools, integrating ecosystem protection into standards, and rolling out resilience initiatives. Monitoring and evaluation will ensure the strategy remains responsive to emerging challenges and opportunities.

Conclusion

ASC's Climate Strategy represents a transformative step toward a climate-resilient aquaculture sector. By focusing on transparency, ecosystem protection, and adaptivity, the strategy provides a clear, actionable framework for producers and supply chains to understand their climate impacts, mitigate risks, and build resilience. This presentation will outline the strategy's development, pillars, and next steps, inviting collaboration to accelerate climate action in aquaculture.

BIODIVERSITY AND ECOSYSTEM FUNCTION ASSOCIATED WITH MANAGED NATIVE AND PACIFIC OYSTER CULTURE SYSTEMS IN CORK HARBOUR, IRELAND

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Introduction

Oyster habitats are recognised as important biogenic ecosystems that enhance biodiversity, ecosystem functioning and coastal resilience through habitat provision, nutrient cycling and benthic–pelagic coupling (Beck et al., 2011). Native European oyster *Ostrea edulis* restoration and Pacific oyster *Crassostrea gigas* aquaculture are increasingly promoted as nature-based approaches to support ecosystem recovery and sustainable food production, yet comparative ecological assessments of these managed systems within Irish estuaries remain limited (zu Ermgassen et al., 2012). Oyster habitats enhance biodiversity by increasing habitat heterogeneity and providing settlement substrate and refuge for diverse faunal and floral assemblages (Theuerkauf et al., 2021). This study investigated biodiversity, trophic structure and functional trait composition associated with commercially managed subtidal *O. edulis* beds and intertidal and subtidal *C. gigas* culture systems in Cork Harbour, Ireland, to evaluate their contribution to ecosystem functioning and nature-based coastal management.

Materials and Methods

The study was conducted at Rossmore Oysters, Cork Harbour, a temperate estuarine system supporting long-standing shellfish aquaculture. Native *O. edulis* habitats included a fallowed post-harvest bed and actively seeded 2+ and 4+ year subtidal beds, while *C. gigas* habitats comprised intertidal bag-and-trestle systems and subtidal beds of differing ages at Jamestown. Biodiversity surveys were conducted using timed visual encounter surveys and replicated dredge sampling. Sampling encompassed multiple habitat types representing different cultivation methods and successional stages. Species were identified to the lowest practical taxonomic level and classified according to trophic and functional traits including feeding mode, mobility, habitat association and structural role. Functional diversity and trophic structure were assessed to examine how biodiversity and ecological functioning developed across differing management and successional stages.

Results

Managed *O. edulis* habitats supported 67 associated taxa comprising 46 faunal species and 21 macroalgal species, while *C. gigas* habitats supported 58 taxa including 46 faunal species and 12 macroalgal species. Oyster-associated habitats consistently supported substantially greater biodiversity than oyster- and shell-free sediments, demonstrating the importance of shell-associated habitat complexity. Functional analyses revealed strong representation of filter feeders, predators, grazers and detritivores across both oyster systems, indicating high functional redundancy and resilient benthic–pelagic coupling. Functional trait composition remained broadly consistent across management stages despite differences in species richness, indicating maintenance of key ecological functions. Older managed habitats generally supported greater trophic complexity and faunal richness; however, younger seeded beds also demonstrated rapid development of diverse assemblages, indicating early establishment of structurally complex communities. Several species of high conservation value were associated with oyster habitats, including the critically endangered European eel *Anguilla anguilla* and egg cases of the endangered spotted ray *Raja montagui*, highlighting the wider ecological value of managed oyster habitats. Commercially and ecologically important taxa including queen scallop *Aequipecten opercularis*, common cockle *Cerastoderma edule*, common prawn *Palaemon serratus* and diverse Rhodophyta assemblages were also recorded.

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Discussion

Managed oyster aquaculture and restoration habitats function as biodiversity hotspots that support resilient ecological communities within temperate estuaries. Shell-associated habitats appear to support diverse assemblages even following harvest or in the absence of living oysters, highlighting the ecological importance of retained shell substrate and habitat complexity (Forrest et al., 2009; Commito et al., 2008). Rotational seeding practices and prolonged *in situ* grow-out allow oyster habitats to develop structurally and functionally diverse biological communities, demonstrating that commercial oyster production and biodiversity conservation can be mutually supportive objectives within ecosystem-based coastal management. These findings demonstrate that well-managed oyster culture can simultaneously support seafood production, biodiversity conservation, ecosystem functioning within working coastal landscapes and contribute to EU restoration policy objectives.

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SEAWEED-DERIVED BIOCOMPOUNDS ENHANCE BROODSTOCK CONDITIONING, REPRODUCTIVE PERFORMANCE AND PATHOGEN RESILIENCE IN THE EUROPEAN FLAT OYSTER *Ostrea edulis*

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Introduction

Hatchery production of the European flat oyster *Ostrea edulis* remains constrained by prolonged broodstock conditioning, inconsistent spawning, disease and limited seed availability, restricting aquaculture expansion and restoration efforts (Lynch & Culloty, 2022; zu Ermgassen et al., 2023). Seaweed-derived functional ingredients offer a sustainable strategy to enhance broodstock performance. Fucoidan and κ -carrageenan, sulphated polysaccharides extracted from brown and red macroalgae, together with selenium, possess recognised immunostimulatory, antioxidant and reproductive health-promoting properties (Li et al., 2008; Rudtanatip et al., 2018; Lynch et al., 2021). This study evaluated, for the first time, the effects of these functional ingredients on broodstock reproductive development, fecundity and pathogen prevalence during hatchery conditioning of *O. edulis*.

Materials and Methods

Two age cohorts of the *O. edulis* (2- and 3-year-old broodstock) were conditioned over a 10-week laboratory trial under hatchery conditions using gradually increasing water temperatures (14–18°C). Twenty-four 12-L tanks containing 30 oysters per tank were maintained. Oysters were allocated to one of four treatment groups: control, fucoidan (*Fucus vesiculosus*), κ -carrageenan (*Chondrus crispus*) or selenium, with three replicate tanks per treatment and age cohort. Treatments were administered weekly for 10 weeks at doses of 150 μg fucoidan g^{-1} whole wet weight, 300 μg κ -carrageenan g^{-1} whole wet weight and 167 μg sodium selenite L^{-1} tank water. Weekly samples were analysed. Broodstock reproductive development was assessed by histological examination of gonadal tissue using the gametogenic staging system of da Silva et al. (2009), together with fecundity estimates based on egg and sperm follicle abundance. Oyster health was evaluated through survival, multiplex PCR screening for *Vibrio* spp. (*V. aestuarianus*, *V. splendidus*, *V. alginolyticus* and *V. anguillarum*), PCR screening for *Bonamia ostreae*, and heart smear examination.

Results

By Week 5, fucoidan- and κ -carrageenan-treated oysters exhibited reproductive development comparable to wild broodstock, indicating accelerated maturation under hatchery conditions. Treated oysters showed a greater frequency of advanced gametogenic stages, increased reproductive activity and enhanced sex switching, while κ -carrageenan produced the highest fecundity. Fucoidan consistently resulted in the lowest cumulative mortality, whereas controls exhibited the highest mortality, particularly in the older broodstock cohort. *Vibrio* spp. prevalence declined from 68.3% in initial broodstock to 25.0%, 15.0%, 11.7% and 13.3% in the control, fucoidan, κ -carrageenan and selenium treatments, respectively. *B. ostreae* prevalence similarly decreased from 76.3% initially to 45.0%, 28.8%, 28.3% and 40.7%, with the greatest reductions observed following fucoidan and κ -carrageenan supplementation.

Discussion

Reliable broodstock conditioning remains a major constraint to the hatchery production of *O. edulis*, limiting consistent seed availability for aquaculture and restoration programmes. These findings demonstrate that macroalgal-derived biocompounds can reduce broodstock conditioning time and associated energy costs while improving spawning reliability. Accelerated maturation has the potential to reduce hatchery operating costs by shortening conditioning time while improving spawning reliability and larval production. The accelerated progression of gametogenesis and increased fecundity observed in treated oysters suggest these sulphated polysaccharides may improve physiological readiness for spawning, while the reduced prevalence of *Vibrio* spp. and *B. ostreae* indicates additional health benefits consistent with their recognised immunostimulatory properties (Li et al., 2008; Lynch et al., 2021). Although selenium provided some benefits, the strongest and most consistent responses were associated with the macroalgal-derived biocompounds. These

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findings support the use of sustainable seaweed-derived functional ingredients as a nutritional strategy for improving broodstock conditioning, hatchery efficiency and reliable seed production of *O. edulis*. Collectively, these findings support the integration of sustainable seaweed-derived biocompounds into broodstock conditioning protocols to improve hatchery efficiency, reliable seed production and the success of European flat oyster restoration programmes.

Acknowledgements

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BALANCING ENVIRONMENTAL AND ECONOMIC PERFORMANCE IN COMMERCIAL INTENSIVE *Litopenaeus vannamei* FARMING IN INDONESIA

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Introduction

Intensive production of Pacific white shrimp *Litopenaeus vannamei* is a major contributor to global shrimp supply and a strategic commodity for export revenue and rural livelihoods in Indonesia (Henriksson et al., 2017; Asmild et al., 2024). Intensification has increased dependence on formulated feed, aeration energy and infrastructure, raising environmental and economic concerns. Although these dimensions are increasingly assessed, integrated evidence from commercially operating farms remains limited. Such evidence is needed to establish benchmarks under real production conditions, determine whether the same processes drive environmental impacts and production costs, and provide a basis for evaluating future improvements (Cortés et al., 2021). This study presents an integrated environmental and economic assessment of a commercial intensive shrimp farm in Sumbawa, Indonesia, using primary production data to characterise baseline performance, identify environmental and economic hotspots, and evaluate sensitivity to key production and market parameters.

Material and methods

The case-study farm operates 45 lined, semi-closed ponds (1,100 m² each) stocked with specific pathogen-free post-larvae at 150 post-larvae m⁻² over two cycles per year (approximately 388 t yr⁻¹). The 104-day reference cycle yielded 4,314 kg pond⁻¹ (mean body weight 20.8 g, survival 69%, feed conversion ratio 1.70). Cradle-to-farm-gate Life Cycle Assessment (LCA; ISO 14040 and ISO 14044) and Life Cycle Costing (LCC) shared a common system boundary, functional unit (one tonne of live whole shrimp at the farm gate) and process-group structure *viz.*, infrastructure and equipment, feed, seed, chemicals, probiotics, electricity and fuel, and effluent (and, for costs only, certification). Foreground data came from farm production records, cross-validated against three years of monthly effluent monitoring; background processes were modelled with ecoinvent 3.10 in SimaPro 10.3 and characterised using ReCiPe 2016 Midpoint (H). Costs reflected 2025 Indonesian market prices. Contribution (hotspot) analysis and one-at-a-time sensitivity analysis, quantified using normalised sensitivity coefficients (NSC), were used to evaluate environmental and economic performance.

Results and discussion

The baseline assessment quantified both the environmental and economic performance of the commercial production system (Figure 1). The system was profitable under the assessed market conditions, with a profit margin of 14.9% and a benefit-cost (B/C) ratio of 1.18 (Table 1). Environmental and economic hotspots only partly coincided: feed dominated global warming (49.6%), terrestrial acidification (54.5%) and land use (89.4%) and was also the largest cost component (44.8%), followed by infrastructure (24.0%) and electricity (14.6%), whereas effluent emissions, which do not incur a direct cost in the studied farm, dominated marine eutrophication (85.0%) and water consumption (97.4%). Profitability was substantially more sensitive to farm-gate selling price than to production costs. NSC values for selling price were 2.5–18 times those of any individual cost component, and a 20% price reduction produced negative gross profit and a B/C ratio below one (Table 1), whereas $\pm 10\%$ changes in feed, infrastructure, electricity or labour costs did not compromise viability. Feed quantity (feed conversion ratio 1.53–1.87) was more influential than electricity across most indicators. The scenarios confirmed that effectiveness depended on the objective pursued. Photovoltaic electricity substantially reduced global warming and terrestrial acidification but increased mineral resource scarcity, whereas local feed sourcing delivered only minor environmental gains while reducing production costs and improving profitability.

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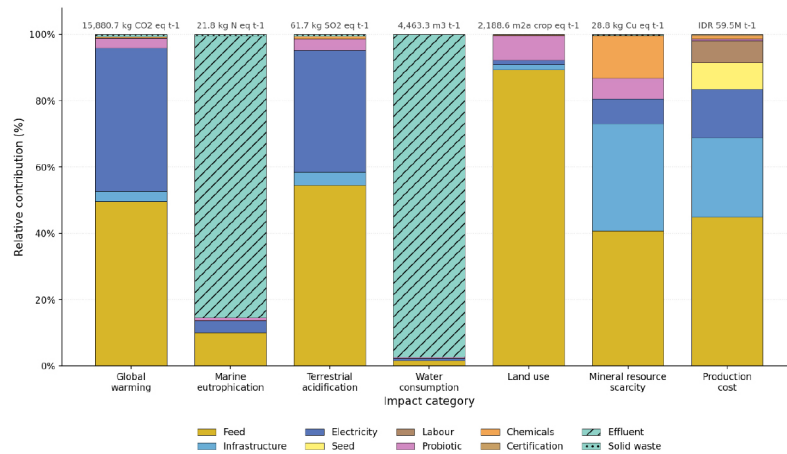


Figure 1. Baseline environmental impacts and production cost of the commercial intensive *Litopenaeus vannamei* farming system in Sumbawa, Indonesia, expressed per tonne (t) of live shrimp produced at the farm gate.

		Total cost (10 ³ IDR t ⁻¹)	Revenue (10 ³ IDR t ⁻¹)	Gross profit (10 ³ IDR t ⁻¹)	Profit margin (%)	B/C ratio (-)
Baseline		59,546	70,000	10,454	14.93	1.18
Selling price -20%		59,546	56,000	-3,546	-6.33	0.94
Selling price -10%		59,546	63,000	3,454	5.48	1.06
Selling price +10%		59,546	77,000	17,454	22.67	1.29
Selling price +20%		59,546	84,000	24,454	29.11	1.41
NSC Selling20			1.00	6.70	5.93	1.00
NSC Selling10			1.00	6.70	5.75	1.00

Table 1. Baseline economic performance of the commercial intensive shrimp farm and sensitivity of profitability to farm-gate selling price, per tonne of live shrimp at the farm gate. IDR = Indonesian rupiah; values converted at a reference rate of IDR 16,400 per USD. Gross profit = revenue – total production cost; B/C ratio = revenue / total production cost.

Conclusions

Environmental and economic performance was driven by different processes and sensitivities. Feed was a shared hotspot for production cost and several environmental impacts, whereas effluent emissions dominated marine eutrophication and water consumption without a corresponding direct farm cost. Profitability was considerably more sensitive to shrimp selling price than to moderate changes in individual production costs. Integrating environmental and economic assessments using commercial production data therefore provides complementary benchmarks for identifying where sustainability priorities align or diverge.

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SUPPORTING AQUATIC ANIMAL HEALTH DATA INTEGRATION IN SALMONID FARMS: A GEOGRAPHIC INFORMATION SYSTEMS APPROACH

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Introduction

Geographic Information Systems (GIS) provide a useful way to bring together farm-level biosecurity measures, antimicrobial usage, and the hydrological context in which salmonid farms are located. In this study, GIS was used to examine how farms are positioned within the hydrographic network and how these positions may be related to possible patterns of water connectivity between establishments. This kind of spatial approach can support a better understanding of issues that are relevant for aquatic animal health (AAH) surveillance.

Materials and methods

The study refers to salmonid farms located in north-eastern Italy, focusing on the Autonomous Province of Trento and the Province of Udine. It integrates data on hydrological network, farm locations, farm-level points of interest (such as water inlets and outlets), biosecurity data, and antimicrobial usage information were integrated into a dedicated geodatabase. PostgreSQL Relational Database Management System with PostGIS and pgRouting extensions were used. GIS-based tools developed in R were applied to analyse water connectivity, exploring hydrological pathways linking farms and their characteristics across the study area.

Results

The application of GIS made it possible to generate maps and spatial outputs that organised biosecurity scores and pharmacological information in relation to farm location and hydrological connectivity, offering useful material for further evaluation by local health authorities (LHA) and decision-makers. The output from the GIS-based tools is provided in GeoPackage, a widely supported file format that allows users to access and use them in commonly available GIS software for further analysis and map production.

Discussion

This work highlights the role of GIS not only in mapping aquaculture systems, but also in combining and analysing heterogeneous information in a more structured way. Bringing together biosecurity, pharmacological and spatial data within the same framework, helped to read farm-level differences in relation to the surrounding hydrological setting. This system is very useful for conducting epidemiological investigations following an outbreak. Although the study was developed for salmonid farms, the same approach could also be adapted to other aquaculture contexts where water connectivity is an important factor.

Conclusion

This GIS-based framework offers a practical way to integrate and analyse farm-level information within a hydrological context. It supports a clearer spatial understanding of farm distribution, hydrological relationships, and data organisation, and may be useful for surveillance-related applications in AAH.

Acknowledgment

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Chlorella vulgaris AQUAFEED SUPPLEMENTATION: FRIEND OR FOE? INSIGHTS FROM A CHRONIC STRESS TRIAL

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Introduction and Aim

The aquaculture industry continues to experience steady growth, with an increased demand for farmed fish, including European seabass (*Dicentrarchus labrax*). However, this growth creates challenges, such as a higher incidence of pathogens and disease outbreaks, which threaten the health and productivity of the sector. Microalgae stand out as a key source of bioactive compounds, showing great promise as functional ingredients in aquafeed formulations. The freshwater microalgae *Chlorella vulgaris* is widely recognized for its rich nutritional profile - comprising proteins, essential amino acids, vitamins, and minerals - supporting its use as a nutrient source for both human and animal consumption. Beyond its nutritional applications, *Chlorella* has been proposed as an attractive, safe, and sustainable strategy to prevent bacterial infections in fish due to its intrinsic antibacterial properties. This study aimed to evaluate the effects of dietary *C. vulgaris* supplementation in European seabass juveniles facing chronic stress and challenged with a common Mediterranean aquaculture pathogen *Photobacterium damsela piscicida*.

Diet composition and Experimental design

A feeding trial was conducted for 4 weeks where *D. labrax* juveniles (~25g) were kept under chronic environmental stress conditions. The fish were fed three different diets: a control diet (CTR) based on commercial formulation, and two experimental diets supplemented with disrupted *Chlorella vulgaris* cells at two inclusion levels (Diet 1: 2% and Diet 2: 4%). Following the feeding period, the fish were infected via intraperitoneal injection with 3.5×10^4 CFU of *Photobacterium damsela piscicida* (MT1415) and mortality was monitored for 8 days. Sampling occurred at baseline (T0, immediately after quarantine and before experimental feeding start), during the feeding period (T1, T2), and at 24h (T3) and 72h (T4) post-infection (hpi). Liver and plasma samples were collected to evaluate oxidative stress (Catalase, LPO, SOD, GSH/GSSG Ratio) and humoral immune parameters (Anti-Protease, Peroxidase, Lysozyme, and Bactericidal activities), respectively.

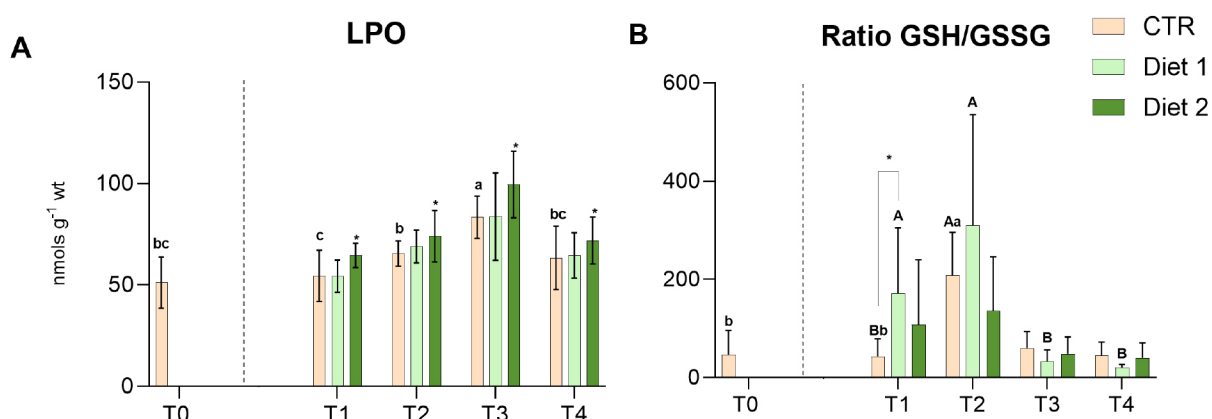


Figure 1. Lipid peroxidation (LPO) levels (nmols.g⁻¹ tissue) and GSH/GSSG ratio in fish fed a control diet (CTR), Diet 1, or Diet 2, sampled at T0 (baseline), T2, T4, 24h post-infection (hpi), and 72 hpi. Bars represent mean $\pm$ standard deviation. Lowercase letters denote significant differences ($p < 0.05$) between sampling times within the CTR group specifically, to allow inclusion of the T0 baseline (present only for CTR). Uppercase letters denote significant differences ($p < 0.05$) between sampling times within the same diet (Time $\times$ Diet interaction). Asterisks (*) denote significant differences ($p < 0.05$) between diets within the same sampling time. The absence of letters or symbols indicates no statistically significant difference. Statistical analysis was performed using a two-way ANOVA followed by Bonferroni's multiple comparisons test.

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Results and Discussion

Exposure to the pathogen significantly altered the physiological and immunological status of *D. labrax* throughout infection. At 24hpi (T3), an acute modulation of the oxidative stress was observed, with a significant increase in lipid peroxidation (LPO) ($p < 0.001$) (Figure 1A) and superoxide dismutase (SOD) ($p < 0.001$) activity, alongside a decline in plasma lysozyme activity ($p < 0.001$). Conversely, at 72hpi (T4), LPO levels decreased to values comparable to baseline (T0) (Figure 1A). Plasma peroxidase and anti-protease activities also showed a marked overall decline post-infection, reflecting the high metabolic cost of exposure to the pathogen while under concurrent chronic environmental stress. Fish fed Diet 2 exhibited higher oxidative damage, with significantly higher levels of LPO than CTR ($p = 0.008$) and Diet 1 ($p = 0.020$) (Figure 1A), suggesting reduced antioxidant protection capacity. The hepatic GSH/GSSG ratio showed a significant Time x Diet interaction ($p = 0.030$) (Figure 1B). The GSH/GSSG ratio of the CTR group peaked at T2 relative to T1 ($p = 0.008$), and this peak also differed significantly from baseline (T0; $p = 0.041$), then gradually declined post-infection to values statistically similar to T0 and T1. In the Diet 1 group, the GSH/GSSG ratio was significantly modulated by the challenge, with a marked decrease at 24hpi when compared to the non-infected animals at T1 ($p = 0.010$) and T2 ($p \leq 0.012$). This parameter also differed between diets at T1, with higher values in Diet 1 group compared to CTR group ($p = 0.010$). However, in this parameter, animals fed Diet 2 showed no significant differences at any individual time point compared with all other diets.

These results indicate that while *D. labrax* possesses a strong capacity of adaptability to cope with a dual-stress scenario (chronic stress and bacterial infection), the dietary supplementation had only a limited effect on most biomarkers analyzed. However, Diet 2 consistently promoted a higher oxidative stress (elevated LPO, stable but comparatively lower GSH/GSSG ratios), suggesting a lower antioxidant protection capacity. Diet 1 presented a more complex profile, although it showed a similar physiological profile to CTR, its redox status did not. The increase GSH/GSSG levels at pre-infection, followed by a marked post-infection collapse, suggests that Diet 1 enhances antioxidant reserves under basal conditions but leaves fish less able to maintain redox balance when challenged. Taken together, these results suggest that the inclusion levels of *Chlorella vulgaris* must be carefully adjusted. Previous studies have reported that prolonged high dietary inclusion of *Chlorella* may impair the fish's recovery capacity rather than providing additional protection.

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SKIN IMMUNE RESPONSES DURING EARLY SALMON LOUSE INFECTION IN ATLANTIC SALMON: COMPARING ATTACHMENT SITE AND NEIGHBOURING SKIN

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Introduction

Research on host–parasite interactions suggests that the exocrine gland of the ectoparasitic salmon louse (*Lepeophtheirus salmonis*), plays an important role in immune evasion, as its secretory ducts may deliver bioactive molecules directly to the attachment site (1,2). In fact parasite-mediated immune suppression at early life stages of the louse in Atlantic salmon (*Salmo salar*) is well documented (3), including inhibitory effects associated with penetration of the salmon skin (4). Concomitantly, these wounds disrupt osmotic balance, elevate stress, and increase susceptibility to secondary pathogens (5). When finally recognised by salmon, the well established parasite induces chronic stress and activates mainly innate, local immune responses (5,6,7). Although several studies have documented this host–parasite interaction comparing immune responses at the attachment site and in neighbouring skin of infected fish, transcriptomic analysis is scarce and have not previously been combined with a proteomic approach.

Materials and Methods

Eighteen Atlantic salmon juveniles (~120 g) were individually housed in 45 L tanks and acclimated for 2 weeks in open-flow seawater (34.08 ± 0.07 ppt; 12.00 ± 0.09 °C). After acclimation, fish were assigned to infected or control groups ($n = 9$ each). For infection, tank water levels and flow were reduced, and *Lepeophtheirus salmonis* copepodids were added at 100 copepodids per fish. Control fish underwent the same handling without copepodid exposure. After 12 days, all fish were sampled. In infected fish, skin was collected from the parasite attachment site (A) and a nearby non-attached skin (NA) for RNA sequencing and proteomic analyses. In controls, skin was collected from the anatomically equivalent region and designated non-infected (NI). Parasite location, life stage and abundance were recorded using a magnifying lens. RNA libraries were prepared with the Illumina TruSeq Stranded mRNA kit and sequenced on a NovaSeq 6000 platform to generate 150 bp paired-end reads. Skin proteins were identified by LC-MS/MS using an Orbitrap Exploris 480. Genes with FDR-adjusted p -values ≤ 0.01 and absolute $\text{Log}_2\text{FC} \geq 2$ relative to NI were considered differentially expressed genes (DEGs), while proteins with $\text{padj} < 0.05$ were considered differentially abundant proteins (DAPs).

Results and Discussion

Twelve days after challenge, salmon lice counts showed that approximately 70% of the parasites were at the chalimus II stage, while the remaining individuals had reached the pre-adult I stage. Transcriptomic analysis revealed a clearly distinct response at the parasite attachment site (A) compared with neighbouring non-attached skin (NA). A total of 35 gene ontology (GO) terms were exclusively enriched at the attachment site (e.g. upregulation of “response to stress”, “regulation of cell population proliferation”, “cellular developmental process”, “regulation of response to stimulus”, “regulation of biological process”, and downregulation of “transcription regulator activity”, “tissue morphogenesis”, “lymphoid lineage cell migration”), whereas only 5 GO terms were exclusively enriched in neighbouring non-attached skin. Only one GO term was shared between the two sampling sites. Interestingly, proteomic analysis revealed DAPs exclusively at the parasite attachment site, with no DAPs detected in non-attached skin compared with skin from non-infected control fish. This pattern further highlights the highly localized nature of the host tissue response to parasite attachment with 12 protein ontology (PO) terms enriched among downregulated proteins (e.g. “wound healing”, “protein hydroxylation” and “cellular oxidant detoxification”) and 64 PO terms upregulated (e.g. “response to stress”, “DNA repair”, “cellular response to DNA damage stimulus”, “pyrimidine-containing compound biosynthetic process”). Importantly, the data collectively confirms that the attachment site is the critical determinant of host response during the chalimus II stage of salmon louse infestation and fundamentally limits the spatial extent of host–parasite interaction, resulting in a response that is predominantly localised to the skin.

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MODULATORY EFFECTS OF DIETARY HALOPHYTE BIOACTIVES IN ATLANTIC SALMON (*Salmo salar*) WELFARE UNDER SIMULATED TRANSPORT

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Introduction

The aquaculture industry faces several challenges leading to a call for novel and sustainable strategies to help enhance animal health and welfare. Within these strategies, functional diets with extracts from halophytes can improve fish health and welfare through the natural antioxidant and anti-inflammatory profile of these plants (Machado et al., 2024). Transport is among the current challenges that farmed fish need to cope with, where the animals are stocked in high densities and subjected to confined spaces. This event is associated as a cause of mortality in commercial species like the Atlantic salmon (*Salmo salar*). Thus, the aim of this study was to evaluate the potential of dietary *Salicornia ramosissima* bioactive compounds in Atlantic salmon stress resilience after a simulated transport, by assessing the physiological and molecular responses to stress induced by this event.

Materials and Methods

Atlantic salmon juveniles (pre-smolt) were hand fed three different diets, a control diet (CTRL), a supplemented diet with 0.5% *Salicornia ramosissima*-derived EXT3 extract (EXT3_0.5%) and a supplemented diet with 0.5% *Salicornia ramosissima*-derived XAD extract (XAD_0.5%) through 26 days. Two RAS systems with a total of 18 tanks were used to distribute the fish into three dietary treatments (CTRL, EXT3_0.5% and XAD_0.5%) in triplicate tanks. After the 26-day feeding period, fish were sampled immediately before the transport (T0h), 2 hours after transport (T2h) and 24 hours after transport (T24h). At each sampling point, fish haematological profile, plasma humoral immune parameters such as peroxidase activity and nitric oxide production as well as plasma metabolites like glucose, lactate and cortisol were analysed. The expression of target genes in the head-kidney and brain was evaluated to obtain a comprehensive assessment of the molecular basis of immune regulation and stress responses.

Results

In response to transport stress, plasma peroxidase activity significantly decreased, with lower values at T2h and T24h compared to T0h. Similarly, blood leukocyte and erythrocyte counts were reduced following transport stress. Systemic effects were also evident from head kidney gene expression analyses, which showed reduced *il12* expression at T24h compared to T0h and T2h, accompanied by decreased *il8* mRNA expression after transport and increased *saa5* and *sod* expression at T24h relative to T0h. In the brain, *mrc3* mRNA expression was significantly decreased in response to stress (T0h in regards to T24h) while *il1b* was more expressed at T2h than at T24h. More importantly, diet significantly affected plasma glucose and lactate levels at T24h, with fish fed the XAD_0.5% diet showing higher concentrations than those fed the EXT3_0.5% diet, although neither treatment differed significantly from the CTRL diet. In the brain, *mrc3* expression was higher in fish fed the EXT3_0.5% diet than in those fed the XAD_0.5% diet. Conversely, *il6* expression was upregulated in fish fed the XAD_0.5% diet compared with the CTRL group, whereas *il1b* mRNA levels were higher in fish fed the EXT3_0.5% diet than in the CTRL group.

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Discussion

This study evaluated the potential of *Salicornia ramosissima* bioactive compounds to improve Atlantic salmon stress resilience to transport. Physiological and molecular responses were triggered by transport whereas the supplemented diets evidenced only moderate effects on the analysed parameters. Transport activated secondary stress responses since plasma glucose and lactate concentrations increased after 24h. The reduction in plasma peroxidase activity suggests a transient suppression of humoral innate immunity, consistent with the immunosuppressive effects of acute stress. Similarly, decreased leukocyte and erythrocyte counts are typical indicators of stress in salmonids and may result from cortisol-mediated redistribution of immune cells and changes in erythrocyte dynamics. Gene expression analyses supported these physiological findings. In the brain, *mrc3* was the only gene significantly affected by transport, showing reduced expression at T24h, which may reflect altered neuroimmune surveillance after transport. The head-kidney gene expression showed higher transcriptional activity particularly in innate immunity and antioxidant defence genes. The downregulation of *il12* and *il8* indicates a temporary suppression of pro-inflammatory signaling, whereas the increased expression of *saa5* and *sod* at T24h suggests activation of the acute-phase response and antioxidant defenses during recovery. While dietary treatments seem to have mild effects, the possibility to improve the potential of these extracts can rely in testing different concentrations of the extracts and other feeding periods. These findings suggest that the two halophyte extracts differentially modulated neuroimmune gene expression, likely due to differences in their phytochemical composition. Despite the limited effects observed in this study, the use of *Salicornia ramosissima* derived extracts as a sustainable functional ingredient for the aquaculture industry deserves further research.

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ZERO FISH OIL DIETS CONTAINING INSECT AND FATTY ACID ETHYL ESTERS OIL IMPROVED SURVIVAL OF EUROPEAN SEABASS UNDER *Vibrio anguillarum* CHALLENGE

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Introduction

Aquaculture expansion increases demand for sustainable feeds that reduce reliance on fish oil (FO). However, reducing dietary FO in aquafeeds is challenging, as FO is a major source of n-3 LC-PUFA, namely EPA and DHA, which marine fish require. Promising alternatives to FO have emerged, driven by their fatty acid composition, cholesterol content, and lipid-soluble vitamin profile. Among these are oil from *Hermetia illucens* larvae (HIO), rich in medium-chain fatty acids, and fatty acid ethyl ester oil (FAEE), a FO-derived by-product of omega-3 concentration production for human consumption, containing approximately 5–8% EPA and DHA[1,2]. This study evaluated the effects of dietary FO replacement by HIO and FAEE on juvenile European seabass (*Dicentrarchus labrax*) growth performance, feed utilization, fillet fatty acid composition, plasma immune parameters, blood cell counts, and survival following a bacterial challenge.

Materials and Methods

Four isoproteic and isolipidic diets containing 48% crude protein and 15% crude lipids were formulated, varying only in lipid source via different combinations of FO, FAEE, and HIO. The positive control (diet D1) contained 78.2% FO and 21.8% HIO, whereas the negative control (diet D2) contained 21.8% FO and 78.2% HIO. Diets D3 and D4 were FO-free and included HIO and FAEE at different proportions (56.4%/43.6% and 21.8%/78.2%, respectively). A feeding trial was performed with European seabass (initial body weight: 25 g) in a thermo-regulated recirculating aquaculture system comprising 12 tanks of 200 L capacity (15 fish per tank) at 23.0 ± 1.0 °C. Each experimental diet was tested in triplicate, and fish were fed until apparent visual satiation for 84 days. At the end of the trial, 3 fish per tank were euthanized by anesthetic overdose, bulk weighed, and blood, plasma, and muscle were sampled. Total red blood cell (RBC) and white blood cell (WBC) counts were determined. After the feeding trial, 20 fish per diet were intraperitoneally injected with 100 μ L of 3×10^7 CFU mL⁻¹ *Vibrio anguillarum* (VA). A control group was injected with 100 μ L of phosphate-buffered saline solution (PBS). Fish survival was monitored for 9 days. Dead or moribund fish were daily removed from each tank. Nine additional fish from each diet were also injected as above, and blood and plasma samples were collected 24 hours after injection for RBC and WBC counts and plasma immune parameter assessment. Statistical analyses were conducted using one-way ANOVA, followed by Tukey's test for pairwise comparisons ($P < 0.05$).

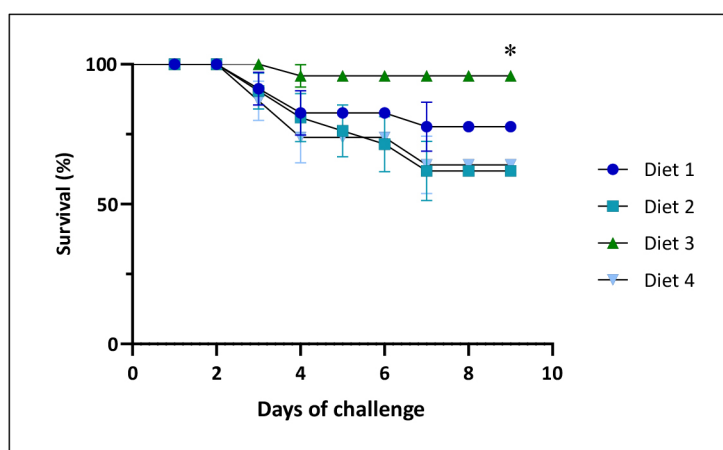


Figure 1 – Kaplan-Meier survival curves during the 9-day bacterial challenge of European seabass fed the experimental diets. Data are represented as mean (n=2) and standard deviation. Significant differences are denoted by asterisks * (p-value = 0.0371).

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Results

No differences in growth performance or feed efficiency were found in fish fed the experimental diets. Plasmatic proteases, anti-proteases, and peroxidase activities were not affected by dietary treatments. Nevertheless, plasma lysozyme activity was higher in fish fed D1 compared to fish fed D2 and D3, but not to those fed D4. Fish fed D3 and D4 showed lower WBC counts than those fed D2, and fish fed D3 had lower RBC counts compared to fish fed D4. The fatty acid profile of the fish fillets is still under analysis. Upon bacterial challenge, no differences in WBC or RBC counts were observed between dietary treatments, and plasmatic immunological parameters are still under analysis. However, fish fed D3 showed higher survival against VA than the other groups (Figure 1).

Conclusion

In conclusion, the present results showed that dietary replacement of FO with HIO and FAEE oil maintained growth and feed efficiency, and that a balanced combination of HIO and FAEE in the diets enhanced disease resistance following a VA infection.

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WHITE GRAPE MARC EXTRACTS AS MODULATORS OF TURBOT IMMUNITY-RELATED GENE EXPRESSION

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Introduction

Aquaculture is one of the fastest-growing food production sectors, and this growth has been followed by increased occurrences of infectious diseases and antibiotic use, contributing to antimicrobial-resistant bacteria and drug residues. In the absence of effective strategies to fully control bacterial pathogens, there is an urgent need for alternatives to control bacterial outbreaks while minimizing impacts on the environment and consumers. In this context, the present study evaluated the use of AquaGrape extracts (glycol (PLN) and ketone (ACA)) derived from white grape marc, a polyphenol-rich byproduct from the wine-making industry with proven antioxidant and antimicrobial properties, as a functional feed additive to assess the potential protective effects in turbot (*Scophthalmus maximus*) upon bacterial challenge with *Vibrio anguillarum* and their immunomodulation capacity.

Material and methods

Five isolipidic (14%) and isoproteic (54%) experimental diets were formulated to meet the nutritional requirements of turbot. A control diet without extract (CTRL) and four diets supplemented with glycol (PLN) or ketone (ACA) extracts at 450 or 900 ppm of total polyphenolic content per kg of feed were tested in triplicate. Groups of 20 fish with an initial mean body weight of 20g were distributed into a total of 15 tanks. Fish were fed for 88 days, after which they were intraperitoneally injected with either 100 µL of *V. anguillarum* (3×10^7 CFU mL⁻¹) or PBS (placebo control). Mortality and clinical signs were monitored over a 12-day period. Gene expression of immune-related genes in the head kidney of nine fish per treatment, before and after bacterial challenge, was analyzed as described by Gonçalves et al. (2026).

Results

Following the *V. anguillarum* challenge, cumulative mortality ranged from 33.3% to 73.3% across treatments. Although no statistically significant differences were observed, the PLN450 group had the lowest mortality (33.3%), followed by ACA450 (46.7%), CTRL (53.3%), PLN900 (60.0%), and ACA900 (73.3%). No mortalities were observed in PBS-injected fish. Gene expression analysis showed that fish fed PLN450 had higher anti-inflammatory cytokine *il-10* and *tgf-β1* expression before challenge when compared to the CTRL diet. After the bacterial challenge, fish fed the PLN450 diet showed lower expression of the pro-inflammatory cytokine *il-1β* compared to those fed the CTRL diet.

Conclusion

PLN extract at 450 ppm was associated with a higher survival rate in turbot and modulated immune-related gene expression, suggesting an enhanced immunological status prior to bacterial challenge and a more balanced response following infection, avoiding excessive inflammation and oxidative stress. Notably, higher doses of both extracts (900 ppm) were associated with higher mortality, highlighting the critical importance of dose optimization when incorporating grape marc extracts as functional feed additives.

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DIETARY FISH OIL-BASED FATTY ACID ETHYL ESTERS SUSTAIN INNATE IMMUNE FUNCTION AND PLASMA BIOMARKERS IN EUROPEAN SEABASS (*Dicentrarchus labrax*) UNDER THERMAL CHALLENGE CONDITIONS

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Introduction

Marine heatwaves are intensifying in the Mediterranean, increasingly constraining the welfare and performance of marine fish. In parallel, the limited availability of fish oil (FO) continues to drive the search for alternative dietary sources of n-3 long-chain polyunsaturated fatty acids (LC-PUFA). Fatty acid ethyl esters (FAEE) are by-products of the industrial concentration of omega-3 fatty acids (FA) from marine oils for human consumption, containing approximately 5–8% EPA and DHA, representing a potentially sustainable LC-PUFA source for aquafeeds. However, their nutritional and physiological adequacy for marine fish under thermal stress has not yet been established. This study assessed the effect of dietary FO substitution with FAEE on survival, plasma metabolites, humoral innate immune parameters, and immune-related gene responses in European seabass (*Dicentrarchus labrax*) juveniles exposed to a thermal challenge.

Material and methods

Four isonitrogenous (48% crude protein) and isolipidic (16% crude lipid) practical diets were formulated with different FO/rapeseed oil (RO)/FAEE ratios (% of total added oil) of 78/22/0 (D1, control), 22/78/0 (D2), 22/56/22 (D3), and 0/22/78 (D4). European seabass (initial body weight 62 g) were fed the diets for 88 days at 23 °C, and then exposed to a two-week thermal challenge (29 °C for one week followed by 31 °C for another week). At the end of the thermal challenge, plasma and head-kidney samples were collected from 9 fish per treatment. Plasma biochemical parameters (glucose, triacylglycerols, total cholesterol, phospholipids, and total protein), innate humoral immune parameters (lysozyme, peroxidase, protease, and antiprotease activities), and the expression of immune-related genes in the head-kidney were determined. Data were analyzed by one-way ANOVA, followed by Tukey's test to highlight differences among groups ($P < 0.05$).

Results and discussion

Cumulative mortality was unaffected by dietary treatment, with no mortality recorded during the first six days of the challenge. Thereafter, final mortality reached approximately 35%, 15%, 35%, and 20% from D1 to D4, respectively. FAEE did not affect plasma glucose, triacylglycerols, cholesterol, phospholipids, or total protein concentrations across groups. Similarly, the innate immune parameters, lysozyme, peroxidase, protease, and antiprotease activities were not negatively affected by FAEE oil dietary inclusion in substitution for FO. The *CD8α* expression was higher in D2 than in D1 and D4 groups, whereas *hsp90* expression was higher in D3 than in D4 groups. No differences between groups in *il-1β*, *tnf-α*, *cox-2*, *nf-κb*, *il-10*, *mhc I*, *mhc II*, *CD4*, *IgM* and *casp3* expression were observed. Overall, the present results indicate that replacing FO with FAEE did not impair fish survival or alter their immunological status during the thermal challenge period. These findings indicate that dietary FAEE may replace FO without adversely affecting immune competence or physiological homeostasis, even under challenging environmental conditions.

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OXIDATIVE STRESS RESPONSES OF EUROPEAN SEABASS (*Dicentrarchus labrax*) FED FISH OIL-BASED FATTY ACID ETHYL ESTERS AS A FISH OIL SUBSTITUTE UNDER THERMAL CHALLENGE

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Introduction

Replacing fish oil (FO) with sustainable alternative lipid sources is a major challenge for aquaculture. Although vegetable oils are widely used in aquafeeds, their low content of n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs) may compromise physiological functions related to immunity and antioxidant defense in marine fish. Fatty acid ethyl esters oil (FAEE), a by-product of FO processing for the human omega-3 supplement industry, retains 4–10% n-3 LC-PUFAs and therefore represents a promising marine-derived alternative to FO. However, thermal stress, as that associated with marine heatwaves, promotes excessive production of reactive oxygen species (ROS), increasing the risk of oxidative damage when antioxidant defenses are overwhelmed. Because dietary lipid sources influence tissue fatty acid composition and membrane susceptibility to lipid peroxidation, replacing FO with FAEE oil may affect the oxidative response of fish exposed to elevated temperatures. Nevertheless, the effects of dietary FAEE oil on oxidative homeostasis under thermal stress remain largely unknown. Therefore, this study evaluated the effects of replacing dietary FO with FAEE oil on the oxidative status and antioxidant responses of the liver and intestine of European seabass (*Dicentrarchus labrax*) exposed to a thermal challenge.

Material and methods

Four isonitrogenous (48% crude protein) and isolipidic (16% crude lipid) diets were formulated with different FO/rapeseed oil (RO)/FAEE ratios (% of total added oil) of 78/22/0 (D1, control), 22/78/0 (D2), 22/56/22 (D3), and 0/22/78 (D4). European seabass (initial body weight 62 g) were fed the diets for 88 days and then exposed to a two-week thermal challenge (29 °C, then 31 °C, one week each). At the end of the thermal challenge, 9 fish per treatment were sampled, the liver and intestine were collected to access total glutathione (tGSH), reduced glutathione (GSH), oxidized glutathione (GSSG) and lipid peroxidation (LPO) concentrations and, the activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione reductase (GR). Data were analyzed using two-way ANOVA with diet and organ as fixed factors, and differences between groups were identified using Tukey's test ($P < 0.05$; $n = 9$).

Results and discussion

Oxidative stress indicators, both non-enzymatic and enzymatic, were unaffected by dietary treatment. No significant diet × tissue interaction was observed, indicating that the dietary effects on the evaluated parameters were similar across the two tissues analyzed. However, differences were observed between tissues. The liver showed higher tGSH, GSH, OSI levels, and CAT and SOD activities, whereas the intestine showed higher GR activity. Overall, replacing FO with FAEE oil did not impair the oxidative status or antioxidant defense system of European seabass exposed to thermal challenge, supporting their potential as a sustainable alternative lipid source in aquafeeds. The absence of dietary effects suggests that FAEE oil maintained redox homeostasis comparably to FO.

Acknowledgment

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TRANSFORMING FISH FARM EFFLUENTS INTO ALGAL BIOMASS: A LOW-COST PHOTOBIOREACTOR FOR IN-SITU ALGAL CULTIVATION WITHIN AN INTEGRATED MULTITROPHIC AQUACULTURE (IMTA) SYSTEM

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Introduction

Microalgae serve as promising raw materials for commercial applications, yet high production costs hinder widespread cultivation. To address this and enhance the sustainability of marine aquaculture, a low-cost floating photobioreactor (PBR) was developed for *in-situ* phytoplankton cultivation within an Integrated Multitrophic Aquaculture (IMTA) system. This system exploits the surplus dissolved nutrients released by fish farms and utilizes natural wave action and solar radiation, significantly reducing the operational expenses typically associated with land-based, closed PBRs. Our objectives were (1) to conduct trials to use this floating PBR type under real-world operating conditions; (2) to demonstrate the ability of the natural phytoplankton communities to grow utilizing the nutrients in the water column next to fish farm's cages, in the absence of various levels of predation; and (3) to identify which planktonic groups are most capable for growing in floating PBRs.

Methodology

The floating tubular PBRs (Fig. 1) consisted of transparent plexiglass tubes fitted with a series of plankton nets (1, 20, and 100 μm mesh sizes) to isolate cultured communities from predators while allowing continuous water and nutrient exchange. Three field experiments were conducted near fish cages in fish farms located in both oligotrophic (Rhodos) and mesotrophic (Malesina) marine environments in Greece. Natural plankton communities, filtered at various sizes to manipulate grazing pressure, were used as inoculums. The growth rates and biomass productivity of pico-, nano-, and micro-phytoplankton were monitored using flow cytometry and inverted microscopy over 8 to 12 days.

Results

Most natural phytoplankton functional groups exhibited positive growth rates inside the floating PBRs. Nanophytoplankton populations—particularly the OraNano group (presumably cryptophytes)—demonstrated the highest specific growth and biomass productivity across all environments. OraNano cells notably increased in cell size, further boosting total biomass production, especially in the oligotrophic site. Concurrently, micro-phytoplankton biomass increased substantially. By the end of the incubations, diatoms dominated the microplankton communities; *Chaetoceros* sp. prevailed in the oligotrophic area, while *Pseudonitzschia* sp., *Leptocylindrus* sp., and *Nitzschia* sp. were most abundant under mesotrophic conditions. The shift toward larger cell sizes and sustained growth highlights the competitive advantage of these taxa when grazing pressure is minimized and aquaculture-derived nutrients are available.

Conclusion

In-situ cultivation of microalgae utilizing aquaculture effluents in floating PBRs is a viable approach to biomass production and bioremediation. While overall biomass yields are currently lower than those of conventional land-based systems, the low-cost approach of our system and the robust growth of commercially valuable diatoms and cryptophytes demonstrates significant potential. Future optimization of PBR design and the introduction of tailor-made microbial consortia could further enhance the sustainability and economic output of marine IMTA systems.

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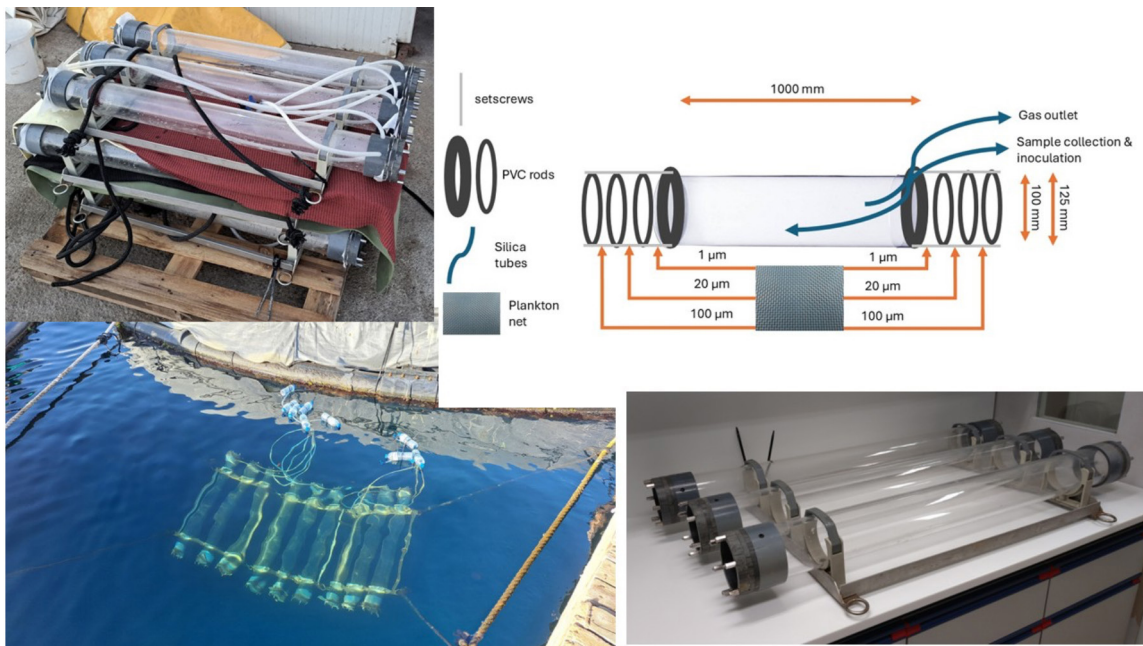


Figure1: The bioreactors design

Acknowledgment

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REGIONAL VARIABILITY OF PHYTOSTEROLS, NUTRITIONAL COMPOSITION AND SENSORY PROFILE IN MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis*) FROM ITALIAN PRODUCTION AREAS

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Introduction

Mediterranean mussels (*Mytilus galloprovincialis*) may show marked differences in edible yield, nutritional composition and sensory profile according to farming area and season. Environmental conditions such as temperature, salinity, trophic status and phytoplankton availability may influence mussel composition and organoleptic quality, with potential implications for product valorisation and market differentiation (Orban et al., 2002; Yaghubi et al., 2021). Particular emphasis is placed on phytosterols, plant-derived sterols structurally similar to cholesterol that are known to reduce intestinal cholesterol absorption by competing with cholesterol during micellar incorporation (EFSA, 2008; EFSA, 2009). Their possible occurrence in mussels, likely linked to phytoplankton intake and local trophic conditions, represents an innovative and still poorly explored aspect in shellfish research, and may contribute to a more functional characterisation of the product (Phillips et al., 2012). The study therefore aims to compare mussels from different Italian production areas through an integrated nutritional and sensory approach, while also evaluating the possible effect of seasonality.

Materials and Methods

The study is starting at the end of May 2026. A first sampling campaign is planned for late spring-early summer, when mussels are expected to show favourable commercial yield, followed by a second campaign at the end of the production season using mussels of comparable size class. According to the current study design, mussels will be collected from up to five Italian production areas: La Spezia, Romagnola (Cesenatico), Sardinia (Oristano), Taranto and Scardovari. The edible portion will be analysed for proximate composition, fatty acid profile, amino acid composition and selected minerals, including selenium, iron, phosphorus, zinc, copper, sodium, potassium and magnesium. Specific attention will be devoted to sterol profiling, with targeted determination of cholesterol and phytosterols in order to explore whether mussels may also represent a source of non-cholesterol sterols of nutritional interest. Sensory characterisation will be carried out by Quantitative Descriptive Analysis QDA[®] with a trained panel using descriptors covering appearance, odour, flavour and texture. Data analysis will compare samples according to production area and sampling season, and explore relationships between sterol profile, overall nutritional composition and sensory attributes.

Results and Discussion

Analyses are currently in progress; therefore, final results are not yet available. Based on the study rationale, we expect to observe area-related and seasonal differences in nutritional composition and specific sensory attributes, reflecting variability among Italian coastal production systems. In particular, differences are expected in edible yield, lipid fraction and mineral composition, as well as in descriptors associated with marine odour, sweetness, juiciness and firmness. Regarding the innovative component of the study, phytosterols are expected to be detected at variable levels among origins and sampling periods, potentially reflecting differences in phytoplankton-derived inputs and local trophic conditions. Their occurrence would be of particular interest because phytosterols are recognised for their ability to interfere with intestinal cholesterol absorption and are therefore associated with cholesterol-lowering potential. The integration of sterol data with nutritional and sensory results may help identify distinctive quality traits linked to origin and season, and support future strategies for product characterisation, communication and valorisation.

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Figure 1. Schematic map of the Italian mussel origin areas included in the current study design.

Acknowledgment

The authors acknowledge the collaborators and mussel producers involved in the sampling activities.

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ELEVATED CO₂ SHIFTS THE n-6/n-3 FATTY ACID BALANCE IN *Nannochloropsis oceanica* DURING THE LATE EXPONENTIAL TO STATIONARY PHASE TRANSITION UNDER DIFFERENT PHOTOPERIODS

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Introduction

Mitigating elevated CO₂ levels in the atmosphere due to anthropogenic sources is at the forefront of this study. The culturing of microalgae can be one of many tools for mitigating these elevated CO₂ levels and its biomass is used in zooplankton culture and early fish and shellfish larval nutrition. Cultures are harvested between the late exponential and early stationary phases when biomass is at its highest, however, lipid and fatty acid profile remodeling also occurs during this period. Studies claim that differing photoperiods may cause changes in the cellular contents of proteins, lipids, and carbohydrates (Khoeyi et al., 2012). Increasing the CO₂ levels in the culture media has shown an enhancement of the lipid content (Jiang et al., 2016; Dubey et al., 2024). Omega-3 polyunsaturated fatty acids (n-3 PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and the omega-6 PUFA arachidonic acid (ARA), are essential in the early development and survival of marine species and the cultured microalgae may supply these necessary biochemical compounds (Matsunari et al., 2013). Therefore, we tested the effects of varying CO₂ levels and photoperiod to determine how CO₂ enrichment and photoperiod influence biomass production and fatty acid composition of the eustigmatophyte *Nannochloropsis oceanica* during the late exponential to stationary phase transition.

Materials and Methods

N. oceanica was cultured using a two-phase semi-continuous system in a 20 L photobioreactor with Walne's medium. Upon reaching late exponential phase, cultures were distributed into 2 L plastic bag photobioreactors. Three independent trials comprising of three photoperiod regimes (24:0, 18:6, and 12:12 Light:Dark) and three CO₂ concentrations (atmospheric 0.04%, 5%, and 10%), each in biological triplicate. Biomass and cellular density were recorded daily. After five days, at the transition to early stationary phase, biomass was harvested for total lipid and fatty acid analysis.

Results

Biomass increased significantly with CO₂ enrichment in the 24:0 h ($F_{2,6} = 8.85$, $p = 0.016$) and 18:6 h ($F_{2,6} = 11.04$, $p = 0.010$) trials, with 5% and 10% CO₂ both yielding significantly more biomass than atmospheric CO₂ in each; the 12:12 h trial showed a similar but non-significant trend ($F_{2,6} = 5.17$, $p = 0.050$). Total fatty acid content followed a comparable pattern across trials. Across all three independent photoperiod trials, elevated CO₂ produced a consistent, dose-dependent increase in the n-6 pathway fatty acid ARA, with the ARA/EPA ratio rising significantly from 0.24-0.28 at atmospheric CO₂ to 0.38-0.54 at 10% CO₂ ($p < 0.05$ in all trials). Furthermore, the entire n-6 pathway was upregulated under higher CO₂ exposure across all photoperiods. This was accompanied by a significant reduction in EPA content in the 24:0 h and 18:6 h trials, evident in both relative and absolute terms. The n-6/n-3 ratio increased 2.0- to 2.2-fold across the CO₂ range in every photoperiod trial tested.

Conclusion

Although elevated CO₂ enhanced biomass production, it consistently shifted fatty acid composition toward higher ARA and lower EPA, increasing the n-6/n-3 ratio regardless of photoperiod. These findings highlight a trade-off between biomass productivity and nutritional quality when culturing *N. oceanica* under high CO₂ in the late exponential and early stationary phases.

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FISH AS FOOD OR FEED? ALIGNING FIFO WITH LCA AND FOOD SYSTEM OBJECTIVES

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Introduction

Aquatic animals provide essential and unique nutrients vital to human health and are produced along a continuum of capture fisheries and aquaculture rather than a simple dichotomy. As fisheries and aquaculture supply chains become under increasing pressures, maximising nutrient output for human consumption is increasingly critical. Marine ingredients, primarily fishmeal and fish oil, have long supported aquaculture production and provided omega-3 fatty acids to consumers, but their use has faced increasing sustainability concerns. Metrics such as the Forage Fish Dependency Ratio (FFDR) and Fish In: Fish Out (FIFO) ratios have been used to assess the use of “fish as feed,” yet they largely overlook nutrient retention from reduction fisheries to final aquaculture products. They also inadequately capture broader food-system dynamics, including market demand, co-product multifunctionality, and the growing use of fish by-product ingredients. Earlier FIFO approaches were not compatible with Life Cycle Assessment (LCA), while later economic allocation (ϵ FIFO) methods improved compatibility but still failed to address nutrient retention. The economic nutrient FIFO (n_{ϵ} FIFO) was developed to quantify marine nutrient retention within aquaculture systems. This paper synthesises evidence on marine ingredient sourcing, evaluates fish-conversion and nutrient-retention metrics, and presents species-specific case studies comparing the various FIFO approaches using a FIFO Performance Tool to support PEFCR compliant LCAs.

Summary of main findings

This section highlights the most important findings of this study, showing challenges and opportunities and where meaningful improvements can be made. These insights point directly to the most effective pathways for advancing progress, towards key Sustainable Development Goals, and re-balancing access to healthy, nutritious seafood globally.

1. Aquatic foods are important for human nutrition and come from a continuum of aquaculture and fisheries. Therefore, it is crucial to maximise nutrient availability for people across the whole system, not treat them as separate silos.
2. Widely used fish dependency indicators like Forage Fish Dependency Ratio (FFDR) and traditional fish efficiency indicators such as Fish In: Fish Out (FIFO) are too narrow and do not consider food system dynamics such as:
 - market demand and competition for fishery resources.
 - multiple end uses of marine ingredients.
 - the increasing role of fish by-products in marine ingredient supply.
3. FFDR and the traditional FIFO are also not compatible with LCA frameworks, they cannot be easily compared with other impact metrics such as Global Warming Potential, limiting their integration in broader sustainability assessment.
4. To consider food system dynamics as outlined in point 2, economically allocated ϵ FIFO offers a more realistic approach considering:
 - treats relative economic value as a proxy for nutritional value and utilisation strategies.
 - assigns higher impacts to limiting feed components (e.g. fish oil).
 - attributes lower footprints to by-products, thereby incentivising their use in aquafeeds.
5. While ϵ FIFO was a significant step forward, it still failed to consider the nutritional content of farmed or feed fish that is a major reason for their inclusion, particularly in regard to critical Long Chain n-3 Fatty Acids (LC n3 FAs).
6. The need for a robust and standardised metric to improve the retention of fishery-derived nutrients (e.g., EPA+DHA) in aquaculture has led to the development of the nutrient Fish In: Fish Out (n_{ϵ} FIFO) metric. This approach, particularly the economically allocated n_{ϵ} FIFO, could help to upcycle hitherto unavailable EPA DHA sources for human consumption
7. An access link to the [FIFO Performance Tool](#) is provided, enabling stakeholders to calculate the different versions of FFDR and FIFO described, to compare with LCA metrics and align with industry standards. This empowers stakeholders to directly apply their own data, integrate the results into broader sustainability assessments, and better evaluate the efficiency and resource implications of different feed strategies (e.g., FCR improvements, higher by-product inclusions, and co-product economic value changes).

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8. Nutrient efficiency is not enough on its own: even if nutrient retention is improved, it does not automatically ensure affordability or access. Nutrition-sensitive strategies are required to guarantee equitable access to key nutrients without overusing environmental resources.
9. Effective fishery management must extend beyond stock status alone to include ecosystem-level assessments and consideration of broader impacts on ecology.

Acknowledgment

We would like to thank Dr. Wenbo Zhang (College of Fisheries and Life Science, Shanghai Ocean University) for providing published papers on Chinese tilapia production systems.

STRATEGIES TO STRENGTHEN EUROPE'S AQUATIC FOOD AUTONOMY

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Introduction

The European seafood market is highly dependent on capture fisheries, while domestic aquaculture, apart from the Norwegian (European Economic Area) Atlantic salmon sector, is lacking scale. Growth capacity is undermined by bureaucracy, industry structure, and cheaper (imported) wild and farmed seafood. Additionally, an estimated 42% of feed ingredients are imported, while aquafeed drives most costs and environmental impact in fed aquaculture value chains. Enhancing European aquatic food autonomy requires system-based policy options. Industry data and linkages with fisheries and agriculture were considered to formulate a resource efficiency framework for European aquaculture. Seafood supply could be enhanced by efficient processing to maximise flesh yield, reclaiming 2 thousand tonnes of EPA+DHA and other nutrients currently discarded, while utilising fish (by-products) without a “food” market in aquafeed. Responsible ingredient sourcing to diversify and grow marine finfish aquaculture should be prioritised, along with co-developing lower trophic (shell)fish species, thereby enhancing the resilience of the European seafood market.

Acknowledgements

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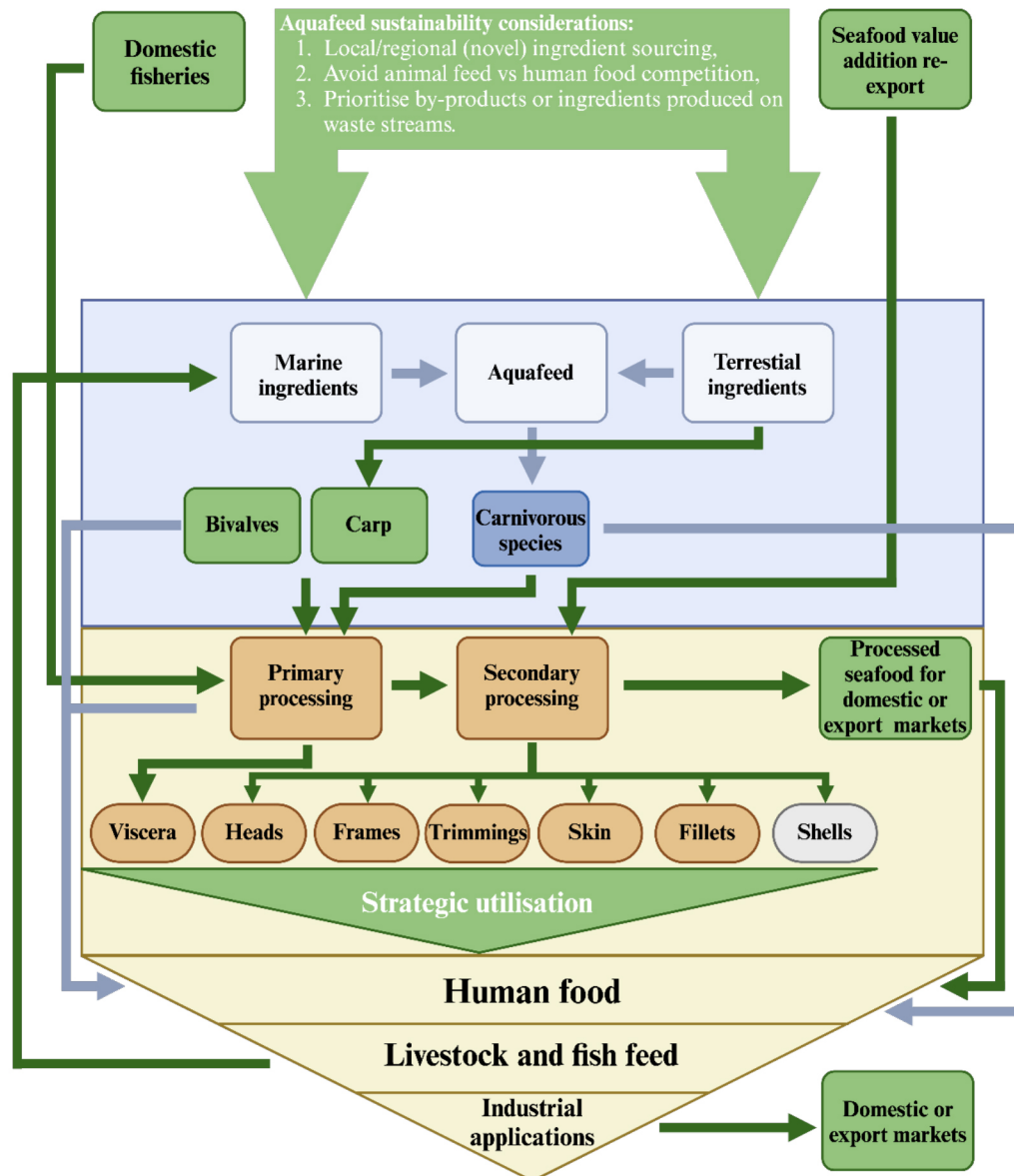


Figure 1: A simplified upstream (blue pattern) and downstream (yellow pattern) aquaculture value chain with opportunities for horizontal expansion (blue) and sustainable intensification (green). Material flow and main farm production in blue, seafood processing and co-products in orange, such as heads, frames (the central skeleton of the fish including the tail), trimmings (fin offcuts), skin (incl. scales), viscera (internal organs) and shells (dark grey) suitable for food, feed or industrial applications. The by-product recovery hierarchy (reversed pyramid) is based on the Stevens et al.⁹ fish recovery hierarchy.

EFFECT OF PRE-RELEASE ADAPTATION ON THE RECOVERY OF NATURAL SWIMMING BEHAVIOUR IN PIKEPERCH (*Sander lucioperca*) BEFORE RESTOCKING

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Introduction

Restocking success depends not only on the number and size of released fish, but also on their functional readiness to survive under natural conditions (Holubová et al., 2025) a mechanism frequently exploited in biomanipulation to stock predator species. Juvenile pikeperch (*Sander lucioperca*). Pikeperch (*Sander lucioperca*) juveniles produced in intensive aquaculture systems may differ from pond-reared fish in swimming behaviour, spatial use, and response to environmental stimuli (Molnár et al., 2018; Turesson et al., 2006). These differences can reduce their ability to adapt after release. Therefore, pre-release adaptation protocols may represent an important tool to restore natural swimming behaviour and improve the performance of cultured pikeperch before restocking.

Materials and Methods

Pikeperch (*Sander lucioperca*) juveniles with an average body weight of approximately 30 g were used in the study. Fish were stocked into two earthen ponds, each with an area of 50 m², at a density of 5,000 individuals per pond. The pre-release adaptation period lasted two weeks. Two adaptation treatments were tested. In the first pond, pikeperch were exposed only to the presence of prey fish. In the second pond, pikeperch were exposed to a combination of prey and predator stimuli. The prey community consisted of native white fish, including roach (*Rutilus rutilus*), while predator stimuli were represented by European catfish (*Silurus glanis*), northern pike (*Esox lucius*), and European perch (*Perca fluviatilis*), with one individual of each predator species present. Swimming characteristics were assessed before and after the two-week adaptation period under controlled experimental conditions. Fish movement was recorded and analysed using computer-assisted image analysis based on EthoVision software. This approach enabled comparison of swimming performance and behavioural characteristics before and after adaptation, and allowed evaluation of the effects of prey-only exposure versus combined predator–prey exposure on swimming behaviour. The experimental design made it possible to separate the influence of prey presence from the additional effect of predator presence.

Results and Discussion

Swimming behaviour of pikeperch juveniles changed significantly after the two-week pre-release adaptation period. Significant differences were observed in total swimming distance, shelter use, maximum swimming speed, and overall duration of swimming activity. Comparison of the prey-only and combined predator–prey treatments allowed us to distinguish the effect of prey presence from the additional influence of predator exposure. These changes suggest that pre-release adaptation modified locomotor activity, risk-related behaviour, and swimming capacity, indicating improved behavioural readiness of pikeperch before restocking.

Acknowledgment

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IMPROVING AQUACULTURE SUSTAINABILITY THROUGH INNOVATIVE FEED INGREDIENTS COMING FROM MACROALGAE AND MARINE INVERTEBRATES

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Introduction

One of the objectives of the Horizon Europe project NOVAFOODIES is to reduce the environmental impact of aquaculture by using more sustainable ingredients replacing traditional fishmeal or use of functional ingredients aiming to improve efficiency, productivity, etc.

Earlier studies have shown that macroalgae can be a promising source of proteins in fish feeds and have interesting functional properties, improving fish health and disease resistance. However, protein digestibility might be a problem limiting inclusion levels. Protein digestibility can be improved by fermentation of the biomass, however thus far experimental research on the use of fermented macroalgal biomass in fish feeds is limited. Macroalgal blooms and beach-wrecked macroalgae count with important populations of small invertebrates as crustaceans, isopods and amphipods feeding on them. Population densities can be extremely, making these also a potentially promising source of feed ingredients.

In this contribution we will present the results of a study testing the effect of four types of prime matter in two different nutritional trials with Atlantic sea bass (*Dicentrarchus labrax*). In a first trial, artificial diets were formulated with either invertebrate meal (3% mixture of small isopods and small crustaceans growing on *Ulva* spp.) as a protein and fatty acid source or meal of the brown macroalga *Ericaria amantacea* (2%) and tested against a control diet (commercial sea bass feed). In the second trial, two levels of inclusion of fermented and non-fermented *Ulva* sp. biomass (5 and 10% respectively), replacing fish meal were tested against the same commercial diet.

All feed ingredients were subjected to rigorous analyses, evaluating proximate composition, detailed fatty and amino acid profiles, biosafety analyses (e.g. heavy metal levels, etc.) and functional properties (e.g. antioxidant activity, anti-microbial activity). After extrusion, physical properties of the feeds were analysed (e.g. density, deformation load, etc.). During the nutritional trials, diet effect on fish performance (growth, health indicators) was evaluated and after the trials, blood samples were taken from the fish to study immunological indicators.

Results and discussion

First trial: invertebrate mixture and *E. amantacea* meal. The invertebrate mixture was characterized by their high protein (35-40% dw) concentration, whereas protein content of *E. amantacea* was considerably lower (7%). Fatty acids were relatively low (4-5 % dw), however, they were in majority poly- and monounsaturated fatty acids.

E. amantacea showed important antioxidant activities, most likely related to their high content in polyphenols. Both invertebrates and *E. amantacea* demonstrated significant bacteriostatic activity on fish pathogens *Photobacterium damsela* subsp *piscicida* and *Lactococcus garvieae*, limited effects on *Vibrio anguillarum* and *Aeromonas veronii* and no significant bacteriostatic effect on *Tenacibaculum maritimum*.

Fish fed the invertebrate or *Ericaria* diet showed no significant differences in growth rate or feed conversion compared to the control diet, as could be expected considering the limited addition. With respect to immunological parameters, both experimental diets showed significantly increased antioxidant activity compared to the control diet. The results of the first trial showed that invertebrate biomass in particular had important nutritional and functional properties that merit further studies to evaluate incorporation in fish feed as a substitute for fish meal.

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Trial 2: non-fermented and fermented *Ulva* sp. biomass grown in nutrient rich IMTA systems. Fermentation process was optimized earlier in the NOVAFOODIES project. Fermentation significantly increased protein content of the *Ulva* (from 37 to 45% dw) and lipid content (from 0.13 to 0.66% dw). Both non-fermented and fermented *Ulva* showed bacteriostatic effects on all five pathogen strains tested. Effects were generally slightly stronger for fermented *Ulva* and the most pronounced effects were found for *Tenacibaculum maritimum* and *Lactococcus garvieae*. Preliminary effects of the fish trials indicate slightly reduced growth at the highest *Ulva* inclusions, however final results are needed for a definite conclusion. After the finishing of the nutritional trials, fish will be subjected to a physiological stress test and a bacterial challenge.

Acknowledgments/Funding

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MAPPING BIOMASS SOURCES OF CRUSTACEAN RESIDUES AND MACROALGAE FOR THE DEVELOPMENT OF HIGH-VALUE FOOD AND FEED INGREDIENTS

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Introduction

The MARMAD project aims to create a sustainable and innovative bio-based value chain for turning marine biomass into high-value food and feed ingredients. The aim is to valorize crustacean residues (VC1: shrimp and blue crab shells) and seaweed (VC2) to develop affordable, nutritious, safe, and sustainable ingredients.

As a first step, a comprehensive analysis and mapping was made of major biomass sources in Europe of these two value chains. For VC1, major crustacean processing plants and biomass flows regarding crustacean biomass processed in the EU have been identified, whereas for VC2, the potential for cultivating and harvesting seaweed across Europe have been examined. This exploration will help to understand how seasonal variations impact seaweed supply and identify the best locations for sourcing. Specific attention has been paid to distribution and harvesting potential of invasive species as the blue crab (*Callinectes sapidus*) and the brown alga *Rugulopteryx okamurae*. Results of these studies are being presented in a series of maps identifying key biomass hotspots.

The analysis combines official statistics, market reports, scientific literature, stakeholder engagement and bottom-up operator mapping. A key methodological principle is that biomass availability depends not only on production or trade volumes but on where physical processing occurs and residues are generated.

Results and discussion

VC1 – Crustacean Residues.

Shrimp residues, Norway lobster residues and emerging crab biomass were identified as the main crustacean resources. Conventional crustacean flows relevant to EU processing amount to approximately 500,000–550,000 tonnes per year (live-weight equivalent), corresponding to an estimated 100,000–110,000 tonnes of potential exoskeleton-rich residues. These figures represent theoretical potential rather than directly available biomass.

The mapping reveals that residue generation does not necessarily follow trade patterns. Spain and Italy emerge as the most important EU hotspots because they combine Mediterranean shrimp fisheries, imports of warm-water shrimp and strong seafood-processing sectors. France functions as both a processing and market node, while Greece is particularly relevant for Mediterranean wild shrimp resources.

Cold-water shrimp supply chains are highly transnational, where Denmark is the principal EU gateway for *Pandalus borealis*, and the Netherlands and Germany are key nodes for *Crangon crangon*. However, a significant share of peeling operations takes place in Morocco (estimated export of 14,000 tonnes corresponding to an estimated amount of 3,000 tonnes residue).

Emerging biomass opportunities from invasive and underutilised crustaceans includes the Northern Adriatic as the main hotspot for blue crab (*Callinectes sapidus*), with around 2,000 tonnes managed in Veneto during 2024. The Adriatic sand crab (*Liocarcinus depurator*) is recognised as a complementary bycatch-based biomass stream.

VC2 – Macroalgae

The macroalgae assessment covers harvested seaweeds, cultivated seaweeds, aquaculture-related green algae and biomass from the invasive alga *Rugulopteryx okamurae*, focusing on six priority species identified in the project, representing brown, red and green algae sourced from commercial production, aquaculture by-products and invasive biomass streams.

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Norway is by far the largest seaweed-producing country in Europe, accounting for more than 50% of total production. France, Ireland and Iceland are the next most important producers. More than 95% of European seaweed biomass currently originates from wild harvesting rather than cultivation.

Brown kelps (*Laminaria* spp., *Saccharina latissima*, *Ascophyllum nodosum*, etc.) dominate production volumes. Major red algae mainly include *Palmaria palmata*, *Gelidium* spp. and *Gracilaria* spp., while green algae production is largely limited to *Codium* and *Ulva* species. Major applications include phycocolloid extraction, animal feed and human food products.

The mapping further shows that production and processing locations are often geographically separated. The invasive alga *Rugulopteryx okamurae* is spreading rapidly across the Iberian Peninsula, with the Strait of Gibraltar identified as the main biomass hotspot.

Overall, the deliverable establishes a strategic foundation for MARMADE's biomass sourcing activities by identifying priority biomass streams, processing hubs and collection hotspots that can support future marine biorefinery operations.

Acknowledgments/Funding

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ACCELERATING DIGITAL TRANSFORMATION IN AQUACULTURE: IMPLEMENTATION OF THE BLUE SHUTTLE INNOVATION PROJECT IN SPAIN

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Introduction

Digitalisation is increasingly relevant for improving the competitiveness, efficiency and resilience of aquaculture businesses. However, the adoption of digital technologies by SMEs is often constrained by limited digital skills, difficulties in identifying suitable solutions and a lack of connection between technological providers and sector-specific needs. BLUE SHUTTLE, an Interreg Atlantic Area project, addresses these challenges through a transnational Blue Innovation Network based on a quadruple-helix approach, connecting industry, research, public authorities and civil society.

Methodology

The project combines stakeholder engagement, capacity building and pilot experimentation to facilitate the adoption of digital solutions in the blue bioeconomy. CTAQUA leads the regional stakeholder-group activities and contributes specifically to the identification of digitalisation needs, the design and delivery of training activities, and the implementation of an aquaculture pilot. Four regional stakeholder meetings have been organised to identify sector needs and assess training priorities. In Spain, CTAQUA delivered three Blue Skills Classroom webinars covering IoT and sensor technologies, data governance and artificial intelligence, followed by a practical workshop focused on developing a digitalisation roadmap for aquaculture companies.

Results and conclusion

The stakeholder engagement activities involved 37 organisations across the project's quadruple-helix network, while the Spanish training programme reached 91 participants. The training activities identified key requirements for effective digitalisation, including reliable data acquisition, connectivity, data governance, interoperability and the development of AI-ready datasets. The process subsequently moved from capacity building towards technological experimentation through a challenge-based innovation approach.

Within the aquaculture sector, launching an Open Call for challenges CTAQUA identified the management of great cormorants at aquaculture facilities as a relevant operational challenge. Following an evaluation process of an open call for digital solutions of this topic, a technology provider was selected to develop and test a prototype based on unmanned vehicles (UGVs) for cormorant management. The pilot is currently under development, with its technological performance, market feasibility and scalability to be assessed during the final stage of the project.

The BLUE SHUTTLE experience demonstrates the value of combining stakeholder-driven needs assessment, digital capacity building and pilot experimentation to accelerate technology adoption in aquaculture SMEs. We have shown how technology transfer can move beyond training activities towards the identification and testing of solutions addressing specific operational challenges. The final assessment of the pilot will provide further evidence on the potential of autonomous technologies as a practical tool for aquaculture management.

Acknowledgments/Funding

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EPA+DHA SENSITIVE AQUACULTURE IN TEMPERATE EUROPEAN CARP PONDS: PROOF-OF-CONCEPT OF BALANCED POND FEEDING STRATEGY

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Introduction

Landlocked Central Europe suffers from extremely low dietary omega-3 long chain polyunsaturated fatty acid (n-3 LC-PUFA) intake, specifically EPA+DHA. Pond-farmed carp (*Cyprinus carpio*) upcycle the limited EPA+DHA from the natural food web in freshwater ponds but can also endogenously fortify their concentrations under the temperate climate. The traditional grain-fed ponds produce carp fillets with low EPA+DHA, while feeding fish meal-fish oil at a high inclusion have profitability, sustainability, and water quality issues. Therefore, we aimed to validate the recently developed balanced pond feeding strategy for European fishponds to circumvent these problems, by using our pond fish nutrition supply-demand model as a guiding tool. The study had the following objectives: (1) a controlled *in vivo* nutritional bioenergetics trial comparing a formulated precision pond feed designed to maximize LC-PUFA retention against a wheat-fed control, and (2) a full-season pond trial using the precision pond feed under balanced pond feeding strategy as a proof-of-concept.

Materials and Methods

Laboratory trial: The “Pond feed” (PF) (31% protein, 9.80% lipid, 0.45% alpha-linolenic acid: C18:3n-3 or ALA, 2.61% linoleic acid: C18:2n-6 or LA, 5.33% saturated + monounsaturated fatty acid or SFA+MUFA, 33% non-starch polysaccharide or NSP, 20% Starch) was formulated via WinFeed using 70% low-opportunity-cost-biomass (corn DDGS, rapeseed and sunflower expeller meal, <5% freshwater fishmeal) and 30% food-feed conflict ingredients (whole wheat meal, rapeseed oil), meeting 90% of the ALA and LA requirement, with sufficient starch and lipids (1:1) as non-protein energy source to stimulate omega-3 sparing effect and higher LC-PUFA retention. This was compared against a traditional wheat control (WF) (9.5% protein, 1.80% lipid, 0.04% ALA, 0.69% LA 0.80% SFA+MUFA, 67.9% starch, 14.3% NSP) in a 70-day bioenergetics trial using common carp (*Cyprinus carpio*; ~20 g initial weight) in a Guelph-RAS system (8 tanks, 23–27 fish/tank; n=4/treatment), fed at 4% body weight/day in two rations. Uneaten feed and feces were collected to quantify actual consumption and whole-body fatty acid mass balance (retention, -oxidation, desaturation/elongation), via initial/final carcass and feed lipid/FA analysis. The trial ended at 70 days, once one treatment tripled its initial body weight. Whole-body fatty acid balance method was applied to understand the turnover of fatty acids *in-vivo*.

Pond trial: a pond validation trial with 2+ age carp (640–660 g) was conducted in 6 ponds at higher stocking density (625 kg ha⁻¹; 975 individuals ha⁻¹). Control ponds (C) (n=3) received wheat May–October; treatment (T) ponds (n=3) received wheat April–June and pond feed (PF) July–October, at equal total dose (~4252 kg⁻¹ ha⁻¹). Water quality and zooplankton biomass (200–500µm) were monitored monthly; harvested fish were analyzed for slaughtering indices, fillet lipid, and FA content. Statistical evaluation was pseudo-replication corrected accounting for the pond identity evaluating a mixed effect of supplementary feeding × pond itself.

Results

Laboratory trial: Both the n-3 and n-6 LC-PUFA biosynthesis rates were significantly higher in the PF than the WF group. In the n-3 LC-PUFA synthesis pathway, Δ5/Δ6 desaturation (PF: 165.6±21.3 > WF: 48.6±39.2 nmol day⁻¹ fish⁻¹), elovl5 elongation (PF: 71.4±7.84 > WF: 23.5±18.5 nmol day⁻¹ fish and elovl2 elongation (PF: 68.6±16.0 > WF: 10.9±11.0 nmol day⁻¹ fish⁻¹) were significantly (*p*<0.05) elevated in PF vs. WF. While both groups synthesized comparable EPA, PF synthesized significantly more DHA than WF (PF: 28.54–38.09 > WF: -4.94–11.17 nmol DHA fish⁻¹ day⁻¹). Concurrently, in the n-6 LC-PUFA synthesis pathway, Δ5/Δ6 desaturation (PF: 173.0±32.8 > WF: 29.3±2.63 nmol day⁻¹ fish⁻¹), elovl5 elongation (PF: 85.7±21.9 > WF: 17.5±2.44 nmol day⁻¹ fish⁻¹), was significantly (*p*<0.05) elevated in PF vs. WF; elovl2 activity was not observed in either group. However, enzymatic activities (elovl5, Δ5/Δ6) toward n-3 and n-6 substrates did not differ significantly within either diet (*p*>0.05). Carp β-oxidized SFA and MUFA for energy, at significantly (*p*<0.05) higher rates in PF vs. WF (SFA; PF: 23.5±3.24 > WF: 3.20±0.20, MUFA; PF: 51.5±2.20 > WF: 9.81±0.62 nmol day⁻¹ fish⁻¹).

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Pond trial:

Gill-filterable plankton biomass did not differ between pond groups during early season ($4.30\text{--}4.43\text{ mg DM l}^{-1}$). However, it diverged significantly during end season (T: 4.97 ± 5.14 > C: $2.24 \pm 2.84\text{ mg DM l}^{-1}$) with the commencement of the application of the precision pond feed in treatment. The whole season plankton consortia FA is under analyses. The final body weight of carp, fillet yield differed between control and treatment ponds, while survivability was the same (>95%). Fillet lipid content did not differ significantly between control and treatment (10.8 ± 0.67 to $10.89 \pm 1.52\text{ g } 100\text{ g}^{-1}$ fillet). Selectively, n-3 LC-PUFA concentrations in fillet were significantly higher in treatment than the control ($p < 0.05$), but n-6 LC-PUFA unchanged ($p > 0.05$). The %RDI of EPA+DHA (recommended minimum intake: $250\text{ mg EPA+DHA person}^{-1}\text{ day}^{-1}$) per 100 g portion size of carp fillets (with skin) was $56.59 \pm 4.57\%$ for control and $83.80 \pm 4.86\%$ for treatment ($p < 0.01$). The yield of EPA+DHA from treatment ponds was $1.607 \pm 0.099\text{ kg ha}^{-1}$, significantly ($p < 0.01$) higher than the control ($0.743 \pm 0.015\text{ kg ha}^{-1}$), equivalent to ~ 4060 and ~ 1883 fish oil capsules $\text{ha}^{-1}\text{ year}^{-1}$, respectively.

Discussion

The carp fed pond feed demonstrated an n-3 LC-PUFA sparing effect by the whole-body fatty acid balance method as well as higher retentions of EPA and DHA than the wheat-fed control group in laboratory settings. In ponds, the in-vivo mechanism was further complemented by presence of a higher natural-food in balanced fed ponds receiving the precision pond feed pellets instead of wheat. Thus, showing a synergistic effect during the end-of-season brought about by the pond feed (*i.e.*, favoring efficient LC-PUFA bioenergetics in carp body) and the boosted natural food web (*i.e.*, favoring enhanced LC-PUFA supply).

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INNOVATION IN AQUACULTURE SOCIAL LICENCE – ‘*Taste the Atlantic – A Seafood Journey*’

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Background

‘*Taste the Atlantic – A Seafood Journey*’ is a initiative of Bord Iascaigh Mhara / Ireland’s Seafood Development Agency, to promote the fantastic aquaculture producers and their products along the internationally recognised ‘Wild Atlantic Way’.

The trail stretches south from wind swept Malin Head in Co. Donegal to beautiful Kinsale in Co. Cork, passing some of Ireland’s most breath-taking seascapes and landmarks - it’s a whole new way to experience the Wild Atlantic Way and to learn more about how Irish seafood is harvested, and the pride, passion and people behind it.

Context

With varying levels of consumer knowledge and acceptance of aquaculture across Europe, this project has highlighted and developed an enhanced social licence and awareness of aquaculture, promoting the many social, economic, cultural and culinary benefits that it provides.

The impact of aquaculture is often under appreciated; income generation, investment and employment, both direct and indirect is often unknown or underestimated by groups and individuals who are not involved directly or indirectly in the industry.

The premium quality and sustainable nature of aquaculture products are not always fully recognized, nor are the significant sustainability efforts that the industry continues to make in animal welfare, elimination of the use of plastics, environmental stewardship and wider social and environmental initiatives.

Social licence to operate (SLO) is critical to ensure the resilience and growth potential of aquaculture.

During 2014, Fáilte Ireland (Ireland’s Tourism Development Agency) launched the Wild Atlantic Way (WAW), a 2,500 Km defined coastal tourism route from Malin Head in the north of Donegal to Kinsale in Co. Cork in the south. The trail is a clearly defined and signposted route that takes in a great a wide part of the rugged west Irish coast.

The opportunity for Irish aquaculture was quickly recognized by BIM as the route was adjacent to many of Ireland’s best aquaculture production areas.

The opportunity to incorporate Irish aquaculture into tourism was quickly grasped and fthe ‘*Taste the Atlantic – A Seafood Journey*’, a defined aquaculture tourism trail was launched soon after.

The development of aquaculture-focused tourism has been successful, and a number of successful ancillary businesses have emerged from existing aquaculture businesses, including numerous oyster farm tours and oyster tasting venues, mussel farm tours, abalone farm tours, coastal foraging opportunities, salmon smokehouse visits, cafes and informal dining experiences.

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Project Impact

The project has had a wide range of benefits, both at an individual aquaculture producer and wider sectoral level.

This includes:

- Increased local and international awareness of Irish aquaculture
- Valuable additional revenue streams, which support aquaculture producers to maintain and build their businesses
- Enhanced and increased employment in rural, coastal locations
- Diversification – being part of the programme has enabled producers to diversify their business, attract new staff, and to bring in valuable revenue streams
- Enhanced profile, professional communications and awareness of the aquaculture sector. A wide range of media features regarding the programme will have been seen by a broad range of stakeholders including consumers, chefs, governmental agencies and the wider public.

The programme has also recently undertaken a strategic review, to assess its impact and importance.

This has created a practical roadmap for its future evolution, to further support aquaculture producers to communicate, promote and champion the importance of the sector.

Acknowledgment

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A PIONEERING PATHWAY TO OCEAN LITERACY & AQUACULTURE SOCIAL LICENCE - THE AQUACULTURE REMOTE CLASSROOM

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Background

Aquaculture is one of the fastest-growing food production sectors globally and plays a critical role in supporting food security, sustainable resource use, and coastal economies. Despite its importance, aquaculture remains underrepresented in formal education and is often poorly understood by the public. Addressing this disconnect requires innovative communication approaches that translate aquaculture science into accessible, engaging, and relevant learning experiences.

The Aquaculture Remote Classroom (ARC) is a state-of-the-art, mobile and expandable classroom positioned on a custom-designed truck. It was developed by Bord Iascaigh Mhara (BIM); The Irish Seafood Development Agency, and co-funded by the Irish Government and the European Maritime, Fisheries and Aquaculture Fund (EMFAF). The unit addresses this need by positioning aquaculture at the centre of a dynamic, mobile education and outreach model.

It manifests itself as a pioneering pathway to ocean literacy. It demonstrates this through the combination of mobility, immersive technology, and curriculum-aligned presentations. Use of the ARC supports the current European ambition to encourage a more ocean-literate society and a resilient blue economy.

<https://bim.ie/a-seafood-way-of-life/the-arc/>

The ARC is highly adaptable, extending beyond schools to engage different audiences through university visits, science festivals, public events and online resources.

High-quality learning tools are used on the ARC such as:

1. Building block activities: demonstrating aquaculture's socio-economic importance
2. Proactive mapping exercises: outlining the best location to farm certain fish species
3. End of day buzzer style quiz - this re-enforces what was taught throughout the workshop.
4. VR Headsets which pupils use to dive beneath the waves and experience aquaculture production up close

These resources have shown themselves to be effective tools that encourage student participation. This setup fosters an action-orientated and collaborative environment allowing students to deeply engage with the learning material provided to them. Take for example, the virtual reality experience which enables ARC users to "dive" beneath the surface, observe aquaculture operations, and explore marine habitats in ways not possible in traditional classrooms.

By offering full-day workshops free of charge and travelling directly to schools across Ireland, the ARC removes traditional barriers to marine education and social licence, ensuring equitable access for rural, coastal, and inland communities alike.

Its content is aligned with the Irish primary school curriculum, supporting teachers in delivering top standard marine and sustainability education from three marine biologists without requiring specialised resources or training.

Impact

In 2025, the ARC engaged 8,290 participants. The unit visited 55 primary schools and 4 secondary schools delivering 112 workshops to 3,837 pupils. 800 ARC participants joined the ARC virtually through an online webinar, which is a condensed version of the workshop deliverable to schools during time periods when the ARC is off the road.

The remaining visitors are made up of members of the public through science festivals, university outreach events, careers fairs, and national marine-themed celebrations. The ARC experience at these events (non-school days) have included shorter hands-on demonstrations, interactive digital content, and facilitated discussions with aquaculture and food sector professionals.

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Collaboration is another key feature of the ARC model. Engagement with marine organisations, industry representatives, educators, and international observers demonstrates its relevance beyond Ireland and its potential as a transferable model for aquaculture communication.

The ARC aligns with national and European policy objectives, including Ireland's strategic priorities for sustainable aquaculture development and broader EU ambitions to build an ocean literate society and resilient blue economy.

By merging marine education, informal outreach, and industry engagement, the ARC showcases a powerful pathway for cultivating an oceanliterate society, building aquaculture social licence and encouraging sustainable coastal futures across Europe.

Acknowledgment

The project is funded by EMFAF / European Maritime Fisheries and Aquaculture Fund.

ASSESSMENT OF CROSS-POPULATION PREDICTION ACCURACY FOR RESPONSE TO COLUMNARIS DISEASE IN TWO DISTANTLY RELATED RAINBOW TROUT (*Oncorhynchus mykiss*) POPULATIONS

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Introduction

Flavobacterium columnare, the aetiological agent for columnaris disease (CD), is a major challenge for several aquaculture species, including rainbow trout (*Oncorhynchus mykiss*). The disease manifests via acute and chronic infections and, leading to high mortality rates, particularly in juvenile fish (Declercq et al., 2013; Evenhuis et al., 2014). Due to the impact of the disease, several vaccines have been developed to enhance CD immune response (Malecki et al., 2021); however, to date no vaccine is commercially available for salmonids. As a result, current practices rely on antibiotic and antimicrobial treatments, posing a risk to both the environment as well as the development of microbial resistance. In response to these challenges, selective breeding for disease resistance offers a viable alternative to improve the sustainability and welfare of farmed populations (Houston et al., 2020). Previous studies on trout CD resistance revealed substantial genetic component (0.20-0.43) and moderate genetic correlation (~0.62) between distantly related populations (Calboli et al., 2022; Fraslin et al., 2022). In addition, performance of genomic prediction on a CD challenge population revealed 18.6% improvement in prediction accuracy compared to application of pedigree-based approaches (Fraslin et al., 2022), highlighting the potential of implementing genomic approaches in selective breeding. While genomic information improved prediction accuracy within a single population, the possibility of assessing disease response using information from unrelated or distantly related populations is currently unknown. The use of previously challenged populations in prediction modelling provides substantial benefits for selective breeding programmes by reducing the need for repeated disease challenge experiments, thereby lowering phenotyping costs and decreasing the number of animals exposed to infectious challenge trials. The aim of the current study was to assess the accuracy of genomic prediction for rainbow trout CD resistance using two distantly related populations.

Materials and methods

Population and genotype filtering

We used publicly available genotype and phenotype information from two commercial rainbow trout populations that participated in a CD survival challenge. Both populations are described in detail in Fraslin et al. (2022) and Calboli et al. (2022). In particular, 1466 fish from the Savon Taimen (ST) population and 2874 fish from the Luke (LK) breeding population with survival records from a *Flavobacterium columnare* challenge trial were genotyped using a 57K Axiom Trout Genotyping SNP array. Genotypes for all fish were filtered using Plink v2 based on the following criteria: minor allele frequency over 5%, SNP genotype call rate over 98%, genotype missingness less than 2% and Hardy-Weinberg equilibrium deviation (p -value $< 10^{-6}$). Finally, the physical coordinates of high-quality SNPs were mapped against the latest version of the rainbow trout genome reference (GCA_013265735.3).

Table 1. Accuracy of genomic prediction models assessed using two genetically distant rainbow trout populations. Within-population accuracy was evaluated using a 10-fold cross-validation approach, whereas in the across-population approach, one population was used to train the model and the second was used for validation.

Training population	Method	Prediction accuracy
Savon-Taimen	cross-validation	0.66 ± 0.01
Luke	cross-validation	0.71 ± 0.01
Savon Taimen	across-population	0.53
Luke	across-population	0.27

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Estimation of genetic parameters

Variance components and heritability for each population were estimated based on genomic relationships using a univariate linear mixed model in AIREMLF90, where binary survival was used as the phenotype, the genomic relationship matrix among candidates was fitted as a random effect and the individual sex and tank (3 classes per population) were fitted as fixed effects. Best linear unbiased predictions for each population were estimated using BLUPF90 and the same linear mixed model was applied in a 10-fold cross validation approach. Cross-population prediction of breeding values was performed with the same model and software as before, using the individuals of one population to train the model and the individuals of the second population for validation. The process was iterated to assess prediction accuracy in both populations. Across all prediction scenarios, prediction accuracy was estimated as the correlation between estimated breeding value and true phenotype, divided by the square root of heritability.

Results and discussion

Quality filtering retained a total of 1459 and 2819 animals for ST and LK populations, respectively, with phenotypes for binary disease survival and genotype information for 22,857 SNPs. Disease survival was estimated at 61.5% and 51.2% for the ST and LK populations, respectively. Estimation of heritability within each population using the cross-validation testing approach showed substantial genetic component (0.20 ± 0.05 and 0.19 ± 0.03 for ST and LK, respectively), consistent with previous findings (Calboli et al., 2022). In addition, prediction accuracy using the same approach was moderately high for both the ST (0.66 ± 0.01) and LK (0.71 ± 0.01) populations (Table 1).

Interestingly, accuracy varied markedly between the two cross-population prediction models. In particular, using the LK population to train the prediction model yielded substantially higher accuracy compared to the model trained using the ST population (0.53 and 0.27, respectively). As the latter population included 1459 all-female individuals (offspring of neo-male female ancestors), differences in prediction accuracy between the two populations could reflect sex-specific effects. At the same time, differences in sample size between the two populations (the LK population contains twice as many individuals) could be additionally contributing to the observed discrepancy in cross-population prediction accuracy. Nevertheless, our study underscores the potential in using distantly related populations to predict CD resistance in rainbow trout. Building on these findings, we are currently investigating the use of whole-genome sequence (WGS) data, functional annotation, and complex genetic variation such as structural variants (SVs) in genomic prediction models to evaluate their potential to further improve across-population prediction accuracy. Results of these analyses will additionally be presented at the conference.

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DIFFERENCES IN THE COMPOSITION OF FATTY ACID PROFILES AND KEY ELEMENTS AND HEAVY METALS IN SELECTED TISSUES OF AQUACULTURE SPECIES OF HIGH ECONOMIC IMPORTANCE

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Introduction

Aquacultured fish are a valuable source of protein for humans, essential minerals, and polyunsaturated fatty acids, particularly long-chain n-3 fatty acids. Their nutritional value and safety depend on the species, diet, environmental conditions, and the ability of individual tissues to accumulate lipids and trace elements. Rainbow trout (*Oncorhynchus mykiss*) and sterlet (*Acipenser ruthenus*) are valuable farmed species, but they differ in physiology, metabolism, feeding patterns, and the distribution of energy reserves, which can lead to differences in their chemical composition. The fatty acid profile of fish tissues is largely shaped by the lipid composition of the feed, but also depends on the species, age, physiological state, environmental conditions, and the function of the organ being analyzed. The liver, as the main organ of lipid metabolism, participates in the synthesis, transformation, and storage of fatty acids, therefore its composition may reflect both the diet and the metabolic capacity of the species. An important element in assessing the consumer quality of fish is the content of essential elements, such as Fe, Zn, Cu, and Mn, as well as potentially toxic metals, including Cd, Pb, and Hg. Their distribution is tissue- and species-specific, and the liver typically accumulates higher amounts of these elements than the muscles due to its role in detoxification and the presence of heavy metal-binding proteins. This relationship has been demonstrated in both rainbow trout and sterlet (Fallah et al., 2011; Jarić et al., 2011). Comparing the fatty acid profile in the liver and viscera and the content of selected elements in the tissues of both species can therefore provide information on their metabolism, nutritional value, safety, and the potential for utilization of by-products generated during fish processing.

Materials and Methods

The study did not require approval from the local ethics committee (LKE) for animal research. The study material consisted of sterlet trout (*Acipenser ruthenus*) with an average body weight of 2.0 ± 0.1 kg, obtained from the Wąsosze Fish Farm near Konin, and rainbow trout (*Oncorhynchus mykiss*) obtained from the Szwarderki Fish Farm (northeastern Poland). The average weight of the studied trout was 0.7 ± 0.05 kg. The liver and viscera were collected from each individual and used for fatty acid profile analysis. The content of selected macro- and microelements and heavy metals was determined in muscle tissue, liver, and viscera. Fatty acid profile analysis was performed using gas chromatography-FID (PerkinElmer; USA). The contents of selected trace elements and heavy metals were determined using ICP-OES (PerkinElmer; USA) and ICP-MS (PerkinElmer; USA) spectrometry, respectively. Statistical analysis of the results was performed using Statistica.13.1 software. The t-test ($P < 0.05$) was used to demonstrate differences.

Results

The tissues (viscera, liver) of the studied species contained, among others, health-promoting unsaturated fatty acids: oleic acid (C18:1 cis 9), linoleic acid (C18:2), α -linolenic acid (C18:3), EPA (C20:5), and DHA (C22:6). In the liver, C16:0, C18:0, C18:1 cis 9, C18:2 cis 9,12, and DHA were detected. In sturgeon liver, significantly higher amounts of palmitic acid C16:0 ($10.81 \pm 0.71\%$) and oleic acid C18:1 cis 9 ($35.88 \pm 4.48\%$) were found. The fatty acid profile of the viscera of both species was dominated by oleic acid C18:1 cis 9, with its proportion being significantly higher in sturgeon ($69.11 \pm 6.43\%$) than in trout ($48.79 \pm 7.69\%$). In trout, the profile was more diverse and included a higher proportion of linoleic acid C18:2, linolenic acid C18:3, and long-chain PUFAs such as EPA and DHA. Studies have shown that the concentrations of heavy metals such as Hg, Cd, and Pb in the analyzed tissues (muscle, viscera, liver) were low and did not exceed the permissible

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levels specified for fish meat in EU regulations. Analysis of the obtained results showed that rainbow trout viscera and liver are a rich source of microelements, especially Fe, Cu, and Zn, which play an important role in metabolic and enzymatic processes. Among the macroelements, potassium dominated in all analyzed tissues, with its highest content recorded in the liver of trout and the muscles of sturgeon.

Discussion/Conclusions

The obtained results indicate that the viscera and liver of the studied fish species can be valuable by-products, particularly due to the presence of health-promoting unsaturated fatty acids such as oleic acid, linoleic acid, α -linolenic acid, and long-chain PUFAs, including EPA and DHA. Of particular significance is the fact that oleic acid dominated in the viscera of both species, while the fatty acid profile in trout was more diverse and included a higher proportion of polyunsaturated fatty acids. This may indicate the potential for using these tissues as a source of lipids with beneficial biological properties. Another important aspect is the low content of heavy metals such as Hg, Cd, and Pb, which in the analyzed tissues did not exceed the permissible levels for fish meat specified in EU regulations. At the same time, the viscera and liver of trout were characterized by high contents of selected micronutrients, especially Fe, Cu, and Zn, with potassium being the dominant macronutrient. The presence of these components indicates that fish processing waste may have not only energy and lipid value, but also mineral value. In summary, the study results confirm that trout and sturgeon viscera and liver should not be treated solely as technological waste, but as a potential raw material for use in the feed, nutraceutical, and cosmetics industries, or in the production of fish oils and mineral supplements. Their use is consistent with the principles of waste reduction and increased efficiency in fish raw material utilization. However, it should be emphasized that further assessment of safety, lipid oxidative stability, and quality standardization of such raw material would be necessary before industrial implementation.

Acknowledgment

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NORWEGIAN SEAFOOD RESEARCH FUND - FUNDING R&D WITH INDUSTRY MONEY – A 25 YEARS' EXPERIENCE

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Introduction

Knowledge is the basis for the development of aquaculture. Late last millennium the Norwegian seafood industry realized that public funding did not fulfill the needs of the industry. An initiative was done to establish a system where all businesses had to contribute. The result was the Norwegian Seafood Research Fund (FHF), funded by a tax of 0.3 percent of export value on all seafood exported from Norway. Over the years since 2001 when FHF was established, more than 2000 small and large projects have been funded, covering all Norwegian seafood industry. The budget has increased from less than 8 mill € in 2001 to 40 mill € in 2025. The projects cover areas from basic biology, ie sequencing the Atlantic salmon genome or microbiota in salmon, to technology focused projects to prevent escapees from fish farms or water quality in RAS systems. Thematic areas have always been decided by the industry, while scientific methodology, evaluation of results, and dissemination of these is the responsibility of the research groups. The basis is “open knowledge”, all industry in Norway, and other countries in fact, can access the results. How was and is it possible, how are the experiences and what results have been achieved? Can the same or similar models be established in other countries to speed up knowledge building? Can this be of help to the total European aquaculture industry?

References

For projects overview – even in Norwegian (FHF is funded by the Norwegian industry) - an open, public database covering all projects is available on [About FHF \(English\) – FHF](#)

AN EXPLORATORY STUDY OF CONSUMER PREFERENCES FOR MUSSELS PRODUCED WITH SUSTAINABLE NET MATERIALS: A CHOICE EXPERIMENT AND PRICE SENSITIVITY ANALYSIS

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Introduction

Seafood products, including mussels, play an important role in food consumption in Europe, especially in Italy (EUMOFA, 2024). In mussel aquaculture, reducing the use of conventional plastics in farming equipment, particularly in nets used during mussel growth, has become an important sustainability challenge (Arantzamendi et al., 2023; Bains et al., 2024). Since many production characteristics of seafood products are not directly observable to consumers, purchasing decisions may also depend on non-visible attributes and external information cues (Carlucci et al., 2015; Cantillo et al., 2021). It remains an open empirical question whether consumers recognise and value these innovations and whether regulatory support for sustainable materials affects their purchasing decisions (Oliva et al., 2019). This study investigates consumer preferences for farmed mussels produced with alternative net materials and tests the role of a regulatory requirement for the use of sustainable materials in mussel farming nets, within the LIFE MUSCLES project (LIFE20 ENV/IT/000570).

Materials and Methods

This exploratory case study analyses consumer preferences for farmed mussels produced with alternative net materials through a Choice Experiment grounded in Random Utility Theory (McFadden, 1974; Louviere et al., 2000). Data were collected in Italy through an online questionnaire distributed via non-probability sampling, yielding 242 completed responses. The questionnaire covered consumption habits, purchasing drivers, sustainability perceptions, label use, and sociodemographic characteristics. Choice tasks required respondents to select among alternatives differing in three attributes: net material (plastic, recycled plastic, bioplastic), the presence or absence of a regulatory requirement mandating a minimum share of sustainable materials in mussel farming nets, and price premiums expressed in €/kg. The regulatory attribute was included to test whether an external institutional requirement linked to otherwise non-observable production practices may influence consumer choice. A no-purchase option was also included to approximate realistic decision contexts (Train, 2009). In addition, the questionnaire included a Van Westendorp Price Sensitivity Meter (PSM) (Van Westendorp, 1976) to explore respondents' perceptions of an acceptable price range for the innovative mussel farming concept as a whole. While the choice experiment was used to estimate trade-offs among specific attributes, the PSM provided complementary evidence on perceived price acceptability.

Results

Results from the choice models show that the sustainability-related attributes are positively associated with the probability of choosing a mussel alternative. In particular, in the conditional logit specifications, both recycled plastic and bioplastic nets display positive and statistically significant coefficients relative to conventional plastic, with the effect of bioplastic being the largest among the product attributes considered. The presence of a regulatory requirement for a minimum share of sustainable materials in mussel farming nets is also associated with a positive and statistically significant effect on choice. By contrast, the price coefficient does not show a stable and robustly interpretable negative sign across specifications. This result suggests caution in deriving direct willingness-to-pay estimates from the choice experiment alone and, more generally, indicates that the monetary dimension is not cleanly identified within the tested price range.

The latent class model points to heterogeneity in preferences across respondents, although these class profiles should be interpreted cautiously given the exploratory nature of the study and the sample size. The three-class solution highlights a larger main group (about 69% of respondents) and two smaller groups (about 17% and 15%), suggesting that respondents differ in the strength and direction of their response to sustainability-related attributes. Additional evidence from the Van Westendorp Price Sensitivity Meter indicates an optimal price point of about €4.85/kg for the innovative mussel farming concept, compared with a reference market price of about €3/kg. This result should be interpreted prudently, as the PSM captures stated price perceptions rather than observed choices; however, it suggests that the price levels used in the choice experiment may have fallen within a relatively acceptable range for many respondents.

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Discussion and Conclusion

For this exploratory case study, the results indicate that respondents tend to prefer mussel alternatives associated with more sustainable net materials, especially bioplastic, and they also respond positively to the presence of a regulatory requirement for sustainable materials in farming nets. These findings are consistent with the idea that non-visible production attributes may matter in consumers' evaluations, although the underlying mechanism cannot be identified directly from the present data.

At the same time, the price attribute does not show a stable and robustly interpretable negative effect across the choice model specifications. This limits the possibility of deriving direct willingness-to-pay estimates from the choice experiment alone and suggests caution in interpreting the monetary dimension of preferences within the tested price range.

In this respect, the Van Westendorp Price Sensitivity Meter provides a useful complementary perspective. Although it captures stated price perceptions rather than observed trade-offs, it identifies an optimal price point of about €4.85/kg, above the reference market price of about €3/kg. Taken together, these results suggest that the surveyed respondents show interest in sustainable mussel farming innovations, while the price dimension requires more careful investigation in future studies based on broader samples and refined price designs.

Acknowledgment

This study was developed within the LIFE MUSCLES project (LIFE20 ENV/IT/000570) (<https://lifemuscles.eu/en/il-progetto/>)

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EFFECTS OF PRE- AND PROBIOTICS ON HATCHING OF *Acartia tonsa*

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Copepods are the natural food source for fish larvae and ideal as live feed in aquaculture. They have an ideal size range and are grown on algae, providing a sustainable alternative to existing aquafeeds of *Artemia* and rotifers, which are nutritionally deficient and can require artificial enhancement with fish meal. The use of copepods is currently limited as they can host fish pathogens and culturing requires a lot of labour and expertise. We tested culturing of the pelagic copepod *Acartia tonsa* with pre- and probiotics and explored potential benefits for commercial production. This is in line with a growing trend in aquaculture towards microbial management. Prebiotics can stimulate a beneficial microbiome and can be converted to short chain fatty acids (pyruvate, acetate, butyrate) with beneficial intestinal effects. Probiotics directly add beneficial microbes and can steer the microbiome in a controlled manner.

Tests were performed in a semi-industrial set-up to test the effect of prebiotic short chain fructo-oligosaccharides, probiotic *Bacillus sp.* and the combined synbiotic treatment. A beneficial effect was observed on hatching success with the prebiotic short chain fructo-oligosaccharides. These fibers are commercially available and cheap, and ideally suited for further exploration. Probiotics were likely administered in a too high dose, and high amounts of dead cell mass were linked to lower hatching success. Treatments with prebiotic fibers (prebiotic and synbiotic treatment) showed a significant change in fatty acid composition and possible de novo lipogenesis under prebiotic exposure.

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CLUSTERS IN BRAZILIAN TILAPIA PRODUCTION: A SPATIAL ANALYSIS

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Introduction

Aquaculture has become one of the fastest-growing sectors in global agribusiness, with farmed aquatic animal production surpassing capture fisheries in total production volume. In Brazil, aquaculture benefits from favorable conditions, including extensive water availability, a suitable climate, and the cultivation of species with strong market potential. Within this context, tilapia farming stands out as the most dynamic segment, accounting for the largest share of Brazilian aquaculture production and serving as the main structural driver of the sector.

Despite its continued expansion, tilapia production is not evenly distributed across the country. There is a strong spatial concentration in specific regions, where the presence of infrastructure, anchor companies, and input suppliers supports the development of specialized production hubs. The central research problem is to understand how this concentration is configured and which factors promote this spatial proximity. The aim of this study is to examine the territorial organization of tilapia aquaculture and identify the determinants that favor the formation of productive clusters in Brazil.

Methodology and Data Sources

The study adopts a quantitative approach based on Exploratory Spatial Data Analysis (ESDA). To assess the geographical organization of Brazilian tilapia farming, the Global Moran's I was used to identify overall spatial autocorrelation; the Local Moran's I (LISA) was employed to identify specific clusters and transition areas; and the Bivariate Moran's I was applied to investigate the relationship between the spatial distribution of tilapia farming and its explanatory variables.

The unit of analysis comprises Brazilian municipalities for the regional analyses and Brazilian states for the national analysis of determinants. The variables were obtained from official secondary databases. The main variable is the share of tilapia farming in total aquaculture production, using data from IBGE/SIDRA for the period from 2013 to 2024.

The explanatory variables include population density in 2022, used as an indicator of demand and obtained from IBGE/SIDRA; public expenditure on science and technology from 2013 to 2024, used as an indicator of innovation and institutional support and collected from IPEADATA; and rural credit variables related to operating costs and investment in agricultural and livestock activities, also covering the period from 2013 to 2024 and obtained from IPEADATA.

The neighborhood structure was defined using spatial weight matrices (W), which were individually adjusted for each region and for the bivariate analyses. The matrices with the greatest statistical power were selected, including k-nearest-neighbor structures with k equal to 3 or 4, in order to ensure greater rigor in the identification of spatial clusters.

Results and Discussion

The analyses confirm that Brazilian tilapia production is characterized by strong spatial dependence, indicating that the performance of a municipality is influenced by the productive activity of neighboring municipalities. Spatial autocorrelation tests confirmed the formation of productive clusters, particularly in the South, Northeast, and Central-West regions. The South presented the highest Global Moran's I (0.552), followed by the Northeast (0.463) and the Central-West (0.385), highlighting the tendency toward spatial concentration of tilapia production in these areas.

Regarding location determinants, the bivariate analysis revealed positive spatial autocorrelation between tilapia production and public expenditure on science and technology ($I = 0.461$), as well as between tilapia production and population density ($I = 0.399$). These findings suggest that institutional support for innovation and proximity to consumer markets are relevant factors in the location and consolidation of the activity in specific areas.

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With regard to financing, a positive spatial association was observed between tilapia production and credit directed toward agricultural activities. Conversely, negative spatial autocorrelation was found in relation to livestock credit ($I = -0.440$), indicating that areas of expanding tilapia production may spatially compete with, or exhibit a distinct specialization from, areas traditionally associated with beef and dairy cattle production. Overall, the results indicate that physical proximity among actors in the production chain generates external economies, facilitates the circulation of technical knowledge, and strengthens regional competitiveness.

Conclusions

This study shows that tilapia production in Brazil is spatially concentrated and strongly influenced by neighboring productive areas. The formation of clusters, especially in the South, Northeast, and Central-West regions, indicates that regional conditions such as infrastructure, technical knowledge, market access, and productive coordination play an important role in the development of the activity.

The results also show that public expenditure on science and technology, population density, and agricultural credit are relevant factors associated with the location of tilapia production. In contrast, the negative relationship with livestock credit suggests that tilapia production follows a territorial specialization distinct from areas traditionally oriented toward livestock activities.

Overall, the findings indicate that policies aimed at strengthening Brazilian aquaculture should support innovation, specialized credit, infrastructure, technical assistance, and coordination among actors in the production chain.

USES OF SATELLITE REMOTE SENSING IN AQUACULTURE: SALMON LICE, HARMFUL ALGAL BLOOMS, MARINE SPATIAL PLANNING SUPORT AND CAGE MAPPING

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Introduction: the Earth Observation for Sustainable Aquaculture (EO4SA) project

Aquaculture offers an additional supply of human food resources as well as an alternative to overfishing. Accordingly, aquaculture is a means to meet the growing global demand for (sea)food and a low carbon footprint protein source, but the sustainability of farming activities is impacted by several key factors, of which four problem areas are being addressed in the four EO4SA Use Cases:

- i. Predicting Risks of Salmon Lice Infestation in Norway.
- ii. Forecasting Harmful Algal Blooms (HABs) Impacting Shellfish Farming in Norway.
- iii. Optimising the Location of Sustainable Shellfish Farming and Tourism in Galicia, Spain.
- iv. Mapping Aquaculture Structures and Use of Marine Resources in Palawan, Philippines

These factors or the environmental conditions associated with them are possible to monitor directly or indirectly by Earth Observation (EO). Therefore, we have developed innovative EO high-level indicators for supporting the sustainable development, regulations and management of aquaculture farming in coastal and offshore waters. This presentation will provide a summary of the project results to date and to collect feedback from the public and private user community for future development of satellite products.

Predicting Risks of Salmon Lice Infestation in Norway

Risk forecasting of salmon lice infestation is a promising EO-based application, leveraging large in situ datasets (>690,000 records) combined with AI models. The project has taken the ocean variables sea surface temperature and salinity, together with the in situ fish farm data, to develop a model to forecast salmon lice outbreaks. A weekly forecast for all Norwegian fish farms is now publicly available, which gives the probability of crossing the lice per fish thresholds that are specified by Norwegian regulations.

Forecasting Algal Toxin Contamination Impacting Shellfish Farming in Norway.

Key environmental conditions from a coastal ocean model, together with a machine learning model to estimate the probability of algal toxins being detected in blue mussels seven days ahead. The forecasted thresholds are above 20 $\mu\text{g}/\text{kg}$ for diarrhetic shellfish toxins – DST and above 120 $\mu\text{g}/\text{kg}$ for paralytic shellfish toxins – PST. The method builds on and extends the published AI model for Lyngen fjord (Silva et al. 2023), now recalibrated using datasets from the entire Norwegian coast (Silva et al. 2024). It is intended that this forecast can help mussel farms in planning mussel harvesting.

Optimising the Location of Sustainable Shellfish Farming and Tourism in Galicia, Spain

The National Park Illas Atlánticas de Galicia (Spain) located at the entrance of the Rías Baixas, four large coastal embayments in the western Galician coast, hosts biodiversity-rich ecosystems and is fully protected, promoting eco sustainable tourism. Visitor numbers are increasing (451,000 in summer 2024, +5% year on year), supporting 26 certified sustainable tourism companies. Although aquaculture is currently prohibited, growing tourism raises interest in identifying surrounding areas for potential touristic expansion or future protection. This case study assessed areas with high mussel growth potential to support tourism-related activities near the park and inform long-term conservation planning.

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Mapping Aquaculture Structures and Use of Marine Resources in Palawan, Philippines

Aquaculture infrastructure is mapped using active and passive multispectral EO data to detect structures such as mussel long lines and fish cages. Building on previous work using a Sentinel 1/2–based method developed for Malampaya Sound (Kurekin et al. 2022), the approach has been extended with artificial intelligence to Puerto Princesa Bay, Palawan (Philippines). A convolutional neural network previously proven in Palawan waters has been retrained and adapted to meet Early Adopters' needs. Historical very high resolution ESA Third Party Mission imagery were used for training and validation of the detection algorithms.

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FROM INVADER TO AQUAFEED: VALORISING *Procambarus clarkii* BIOMASS AS A SUPPLEMENT FOR RAINBOW TROUT BROODSTOCK

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Introduction

Invasive alien species (IAS) represent one of the primary drivers of ecological modification and biodiversity loss in freshwater ecosystems worldwide. Among freshwater invaders, the Louisiana red swamp crayfish (*Procambarus clarkii* Girard, 1852) is recognized as highly damaging due to its extensive burrowing activity, rapid population growth, high environmental tolerance, and pathogens spreading such as the crayfish plague fungus *Aphanomyces astaci* (Gherardi, 2006). To protect native biodiversity and ecosystems in accordance with Regulation (EU) 1143/2014, containment campaigns, which can generate significant quantities of harvested biomass, are imperative. Concurrently, modern aquaculture seeks sustainable alternatives to conventional feed ingredients that are often overexploited (Zarantonello et al., 2022). Processing IAS biomass into functional feed ingredients offers a practical application that aligns with circular economy principles by transforming an ecological threat into a valuable resource. This study, conducted within the Nazzano Tevere-Farfa Nature Reserve (Italy), combined population monitoring with the valorization of captured *P. clarkii* biomass to evaluate its efficacy as a functional, rich in astaxanthin feed supplement for rainbow trout (*Oncorhynchus mykiss*) broodstock. The primary objectives were to evaluate the effects of crayfish meal inclusion on egg biochemical composition, fatty acid profiles, viability, and oxidative status.

Materials and methods

Crayfish fresh biomass harvested in the Reserve (65 kg fresh weight from May 2024 to July 2025) was dried at 70°C (25% moisture) into 1x1 cm cubes (4 kg meal: 14.5% lipids, 39.0% protein, 38.6 mg/kg astaxanthin). An experimental feeding trial was conducted with 24 female rainbow trout at first reproductive age (2.13 ± 0.15 kg) maintained in net enclosures within a medium-hydrodynamic area. Specimens were fed with three dietary treatments at a daily feeding rate of 0.8% body weight for 17 days: a control diet (O, Skretting Optiline), a commercial breeder diet (V, Skretting Vitalis), and an experimental diet (C, Vitalis supplemented with ~110 g of crayfish meal). Over the trial, a total of 3.3 kg of dried crayfish meal was employed. At the end of the feeding period, twenty-one pools of unfertilized eggs from as many females were kept for ROS analysis (8 for O, 6 for V, and 7 for C). Total lipid content was analyzed using standard AOAC procedures, while fatty acid profiles were determined by gas chromatography. Astaxanthin concentration was quantified via HPLC. Reactive Oxygen Species (ROS) levels were measured using a protocol modified by De Iorio et al. (2025). Cellular viability was evaluated using the Neutral Red Uptake (NRU) assay on unfertilized oocytes. The total number of fertilized eggs was 16,943 for O, 22,882 for V, and 24,498 for C. Fertilised eggs were incubated in a continuous-flow vertical system at 11°C and 102% oxygenation, with daily mortality recorded. Statistical analyses were performed using Kruskal-Wallis tests to investigate significant differences between the different treatments. Spearman's rank correlation analysis ($p < 0.05$) was performed to assess whether basal ROS levels were influenced by substrate availability (lipids amount).

Results

Egg lipid content averaged $9.9 \pm 1.4\%$ for O, $9.5 \pm 0.7\%$ for V, and $9.8 \pm 1.0\%$ for C, showing no significant differences among treatments. Across all diets, docosahexaenoic acid (DHA, C22:6n3) was the most abundant PUFA ($9.9 \pm 0.3\%$ in O, $9.8 \pm 0.4\%$ in V, $10.1 \pm 0.4\%$ in C), oleic acid (C18:1n9) was the primary monounsaturated fatty acid ($28.1 \pm 0.6\%$ in O, $29.2 \pm 0.0\%$ in V, $28.5 \pm 0.3\%$ in C), and palmitic acid (C16:0) represented the dominant saturated fatty acid ($12.1 \pm 0.1\%$ in O, $12.5 \pm 0.2\%$ in V, $12.3 \pm 0.1\%$ in C). Arachidonic acid (ARA, C20:4n6) amount changed significantly ($p < 0.05$) from $4.2 \pm 0.6\%$ in diet O to $4.7 \pm 0.1\%$ in diet V and $4.5 \pm 0.1\%$ in diet C. Total PUFA Omega-3 to Omega-6 ratios were comparable across treatments (3.8 for O, 3.5 for V, 3.7 for C). Basal ROS levels and cellular viability showed no statistically significant differences between treatments and groups ($p > 0.05$). Baseline egg incubation mortality showed equivalent trends across groups.

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Discussion and Conclusions

This study demonstrates the feasibility of combining invasive crayfish management with the use of functional aquafeed supplements for rainbow trout broodstock within natural reserves. Although the 17-day feeding period was not sufficient to fundamentally alter the core fatty acid composition of eggs due to maternal somatic lipid legacy (Izquierdo et al., 2001), the significant increase in arachidonic acid (ARA) in treatments V and C highlights its high turnover rate and rapid mobilization into oocytes during final maturation and hydration. ARA plays an essential role in eicosanoid-mediated reproductive signaling and oocyte maturation. Furthermore, dietary supplementation with crayfish meal maintained redox homeostasis, egg viability, and developmental performance without inducing oxidative stress. Consequently, *P. clarkii* meal represents a viable supplementary ingredient that combines invasive species management with circular economy principles in sustainable aquaculture. Future research with extended feeding periods should investigate the potential of *P. clarkii* meal supplement to reduce daily ratio of commercial feeds for broodstock, which represent a particularly costly input for farmers. This approach could be especially suitable for aquaculture facilities located within or near protected areas, where crayfish biomass may be locally sourced by the containment campaigns envisaged under Regulation (EU) No 1143/2014 on invasive alien species offering an alternative end-use for a biomass otherwise destined for costly disposal as special waste.

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THE EFFECT OF FEED SUPPLEMENTED WITH *Sideritis* sp. ON THE MORPHOLOGY AND PHYSIOLOGY OF THE DIGESTIVE AND IMMUNE SYSTEMS OF STERLETS

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Introduction

Sturgeon aquaculture has been growing rapidly, with Poland becoming a significant producer of sturgeon meat and caviar (Elhetawy et al., 2023). Increasing demand highlights the need for optimal rearing conditions and fish welfare in intensive systems. Due to their late maturation and sensitivity to environmental factors, sturgeons require well-balanced diets that strongly influence their growth, development, and reproduction (Szczepański et al., 2025). Intensive farming also increases stress and disease susceptibility, emphasizing the importance of a well-functioning immune system (Di Sotto et al., 2020). With restrictions on antibiotic use and concerns over microbial resistance, natural immunomodulators, particularly plant-based compounds, are increasingly used to support immunity, growth, and survival while reducing environmental impact (Raja et al., 2017). However, their mechanisms and effectiveness in sturgeons are still not fully understood. This study aims to evaluate the effects of selected natural feed additives on the growth, physiology, and immunity of sturgeons in recirculating systems.

Materials and Methods

Sterlet (*Acipenser ruthenus*) juveniles were obtained from a fish farm in Wąsosze (Poland) and reared in a recirculating aquaculture system at the Inland Fisheries Institute in Olsztyn. After a two-week acclimation period, 3-month-old fish (~8 g) were assigned to three dietary treatments: control and diets supplemented with *Sideritis scardica* (2.5% and 5.0%), each in triplicate (30 fish per tank). Fish were fed commercial feed (Nutra T-2.0, Skretting) for 10 weeks under controlled conditions. Growth performance and survival were monitored throughout the experiment, and final body weight and length were measured. At the end, liver and intestine samples were collected for histological (hematoxylin–eosin, Masson's trichrome) and immunohistochemical (proliferation cell nuclear antigen - PCNA) analyses to assess tissue structure and cell proliferation.

Results

No differences in fish growth rates were observed among the studied groups. Morphometric analysis indicated that dietary treatment mainly affected liver structure and cellular proliferation, while intestinal parameters remained largely unchanged. Treated fish showed a reduction in hepatocyte size compared with the control, without clear differences between treatment levels. Most intestinal measurements, including in the anterior and spiral sections, were similar across groups, with only minor and inconsistent trends. The most pronounced effect was observed in PCNA-positive cell area, which was highest in the lower treatment group and lowest in the higher one, suggesting a potential dose-dependent influence on cell proliferation.

Discussion

The present study showed that dietary supplementation with *Sideritis scardica* did not significantly affect sterlet growth, consistent with results for other plant-based additives such as *Echinacea purpurea*, which improved immunity without influencing growth (Almarri et al., 2023). In contrast, some additives (e.g., garlic powder, proteolytic enzymes) may enhance growth, indicating that effectiveness depends on the compound's mode of action (Lee et al., 2014). However, significant differences in hepatocyte morphology suggest physiological responses to supplementation, supported by studies on additives like papain and nucleotides affecting liver function and metabolism (Taklu et al., 2025). Overall, while *S. scardica* did not improve growth, it may influence metabolic processes in sterlet, warranting further research regarding the use of this plant in fish feed.

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EVALUATION OF INSECT-DERIVED LIPIDS AS A DIRECT SUBSTITUTE FOR FISH OIL AS A SUSTAINABLE LIPID SOURCE IN SIBERIAN STURGEON DIETS

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The increasing demand for sustainable aquaculture feeds has stimulated interest in insect-derived lipids as alternatives to fish oil. This study evaluated the effects of replacing fish oil with black soldier fly (*Hermetia illucens*) fat on growth performance, feed utilization, intestinal morphology, intestinal microbiota, economic profitability, and environmental sustainability in Siberian sturgeon. Fish were fed diets containing different ratios of *H. illucens* fat and fish oil (F0–F100). Intestinal microbial communities were characterized by 16S rRNA gene sequencing. Fusobacteriota, Firmicutes, and Proteobacteria dominated the microbiota, while dietary lipid composition significantly influenced the abundance of several bacterial taxa, particularly Clostridia and Bacilli. Growth performance, feed efficiency, and intestinal morphology were also affected by dietary treatment. Among all diets, the 50:50 blend of *H. illucens* fat and fish oil (F50) consistently produced the best overall outcomes, combining superior growth, improved feed utilization, favorable modulation of intestinal microbiota, enhanced economic returns, and improved environmental sustainability indicators. These findings demonstrate that black soldier fly fat is a viable and sustainable alternative lipid source, with partial replacement of fish oil offering the most effective strategy for sustainable Siberian sturgeon production.

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Table 1. Growth performance and feed utilization of Siberian sturgeon fed experimental diets containing BSFL fat.

Parameters	F0	F25	F50	F75	F100	SEM	P-value
Initial BM [g]	99.80	100.20	102.10	100.00	99.00	0.7733	0.1065
Final BM [g]	580.80 ^{bc}	567.00 ^{bc}	624.20 ^a	547.20 ^a	597.00 ^{ab}	9.1370	0.0001
BWG, days 1-60 [g]	481.00 ^{bc}	466.80 ^{bc}	522.10 ^a	447.20 ^a	498.00 ^{ab}	9.1787	0.0001
BWG (per fish) [g]	38.72 ^{bc}	37.80 ^{bc}	41.61 ^a	36.48 ^a	39.80 ^{ab}	0.6091	0.0001
FI, days 1-60 [g]	314.76	310.04	300.66	317.59	303.21	5.0515	0.1236
FCR	0.66 ^{abc}	0.66 ^{ab}	0.58 ^a	0.71 ^a	0.61 ^{bc}	0.0195	0.0008

BM – biomass; BWG – body weight gain; FI – feed intake; FCR – feed conversion ratio; F0 – 100% cold-extracted *H. illucens* larvae fat; F25 – a combination of cold-extracted *H. illucens* larvae fat and fish oil in a 75:25 ratio; F50 – a combination of cold-extracted *H. illucens* larvae fat and fish oil in a 50:50 ratio; F75 – a combination of cold-extracted *H. illucens* larvae fat and fish oil in a 25:75 ratio; F100 – 100% fish oil; SEM – standard error of the mean. Different letters (a, b, c) indicate differences between treatments ($p \leq 0.05$)

Table 2. Intestinal histomorphological parameters of Siberian sturgeon (*Acipenser baerii*) fed diets containing BSFL fat

Parameters	Experimental groups					SEM	P-value
	F0	F25	F50	F75	F100		
Villi height [µm]	774.34	785.68	805.51	897.99	786.68	19.76	0.270
Villi width [µm]	141.17	141.27	145.73	154.97	145.41	2.69	0.494
Villi area [mm]	345.43	352.84	370.68	435.55	362.55	1.22	0.136
Crypt depth	64.62	64.54	66.53	68.16	64.54	1.86	0.960
Muscular layer thickness	113.76	118.20	109.35	116.20	115.18	3.29	0.942

Table 3. Environmental sustainability and economic profitability of Siberian sturgeon fed experimental diets containing BSFL fat

Parameters	F0	F25	F50	F75	F100	SEM	P-value
Environmental sustainability							
FIFO	0.72 ^c	0.77 ^{bc}	0.71 ^a	0.93 ^a	0.84 ^{ab}	0.0243	<0.0001
FCE [g/g]	1.53 ^{bc}	1.51 ^{bc}	1.74 ^a	1.41 ^c	1.64 ^{ab}	0.0444	0.0004
FOU [g/1kg of fish gain]	0 ^a	13.30 ^d	23.06 ^c	42.85 ^b	48.73 ^a	0.9553	<0.0001
FMU [g/1kg of fish gain]	196.67 ^{abc}	199.44 ^{ab}	172.94 ^a	214.23 ^a	182.75 ^{bc}	5.8625	0.0008
Economic profitability							
Group	F0	F25	F50	F75	F100	SEM	P-value
FC [€/kg]	1.84	1.81	1.78	1.75	1.73	-	-
ECR [€/1 kg of fish gain]	1.20 ^a	1.20 ^a	1.03 ^b	1.25 ^a	1.05 ^b	0.0348	0.0004
EPI [€/fish]	3.27 ^{bc}	3.17 ^{bc}	3.64 ^a	3.02 ^c	3.46 ^{ab}	0.0770	0.0001
PRO [€/1 kg of fish gain]	6.80 ^b	6.80 ^b	6.97 ^a	6.75 ^b	6.95 ^a	0.0348	0.0004

FCE—feed conversion efficiency (g/g), FMU—relative fish meal use (g/1 kg of fish gain), FOU—relative fish oil use (g/1 kg of fish gain), FIFO—fish-in fish-out ratio, FC—feed cost (€/kg), ECR—economic conversion ratio (€/1 kg of fish gain), EPI—economic profitability index (€/fish), PRO—profitability (€/1 kg of fish gain); F0 – 100% cold-extracted *H. illucens* larvae fat; F25 – a combination of cold-extracted *H. illucens* larvae fat and fish oil in a 75:25 ratio; F50 – a combination of cold-extracted *H. illucens* larvae fat and fish oil in a 50:50 ratio; F75 – a combination of cold-extracted *H. illucens* larvae fat and fish oil in a 25:75 ratio; F100 – 100% fish oil; SEM – standard error of the mean.

PRODUCTION OF MARKET CARP IN A TWO-YEAR CYCLE – A POSSIBLE REACTION TO CLIMATE CHANGE IN CENTRAL EUROPE!?

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Introduction

Common carp (*Cyprinus carpio* L.) is one of the most important fish species in freshwater aquaculture worldwide (FAO, 2022). It is of great importance in several European countries and will continue to be a significant fish species in areas where it is traditionally farmed. In Bavaria, carps are generally farmed using traditional farming methods in extensive or semi-intensive eathern ponds, with a combination of natural feed sources and supplemental feeding. Due to the traditional culinary preparation of marketable carp in the region of Franconia - called “half baked carp”, marketable carps weighing between 1000 g and 1500 g are in high demand. To achieve this weight, it has traditionally been common practice in Bavaria, given the prevailing climatic conditions, to produce marketable carp in a three summers cycle.

Climate change might be altering the framework for this traditional pond farming in Bavaria. The average annual temperature in Bavaria rose by + 1.9 °C between 1951 and 2019 (LfU, 2022). Species adapted to higher water temperatures, such as *cyprinids*, could benefit from higher water temperatures. Due to current climate change (longer growing periods and shorter winter rest periods), the question arises whether the traditional three summers cycle in Bavarian carp pond farming can be adapted to the changing conditions. Therefore, the aim of this study was to clarify, if carp in Bavaria can be raised to market size in just two summers. This study therefore also aimed to compare the quality of carp meat from carp farmed in two summers- and traditional three summers -cycle.

Material and Methods

The trial on the two-summer production cycle was conducted at the Bavarian State Research Center for Agriculture, Institute for Fisheries, Department for Carp Farming (Middle Franconia, Germany) in 2023 and 2024. The experimental ponds were managed according to traditional pond aquaculture practice, and carps (mirror carps) from the own breeding and own fish stocks (K_1) were used for the two summers production cycle in eathern ponds. To compare carcass and meat quality of two summers old marketable carps, traditional three summers old marketable carps were also produced from the institute’s own K_2 stocks in eathern ponds. Carp stocks were supplementaly fed with pressed triticales (x *Triticosecale* Wittmack) and grounded peas (*Pisum sativum* L.).

To compare the carcass- and meat -quality between two- and three-summers old marketable carps, fish with a nearly the same body weight and fat content were selected. These two summers old marketable carps had average wieght of 1646 ± 181 g and average fat content of 4.0 ± 1.0 % ($n = 20$). These three summers old marketable carps had average wieght of 1638 ± 156 g and average fat content of 4.0 ± 1.5 % ($n = 20$).

Results and Discussion

The K_1 - stocks had average weight of 124 ± 20 g ($n = 150$) within the first production year from K_0 to K_1 in year 2023. The following second production year (2024) of K_1 up to marketable carps in 2024 clearly shows that under current climatic conditions and with traditional pond management in Middle Franconia, it is possible to produce marketable carps in eathern ponds in just two years. These two summers old marketable carps had an average weight of 1700 ± 264 g ($n = 108$).

The results of the carcass examination of selected two summers old marketable carps show a significantly ($p < 0.05$, $n = 20$) higher fillet yield with skin and fillet thickness (excluding skin) compare to selected three summers old marketable carps. Contrarywise the gonadosomatic index (GSI) was significantly ($p < 0.001$, $n = 20$) higher in these three summers old carps than in these two summers old carps. The age has a strong effect on gonad development of the European carp (Hulata et al. 1974). However, differences of the meat quality (connective tissue, firmness, colour, sensory evaluation, cooking- and baking -losses) between these three summers old marketable carps and these two summers old marketable carps were none or marginal.

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BIOACTIVE AMINO ACIDS AS POTENTIAL INDUCERS OF METAMORPHOSIS IN *Ruditapes decussatus* LARVAE

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Introduction

One of the major bottlenecks in bivalve hatchery production is the low efficiency of larval settlement and metamorphosis. These events determine the transition from planktonic free-swimming larvae to benthic juveniles and are associated with high mortality, representing a major challenge for bivalve aquaculture. Several neuroactive compounds, such as catecholamines and neurotransmitters, have been investigated as chemical inducers of larval metamorphosis in bivalves, although their effectiveness is often species-specific and influenced by treatment conditions [1]. More recently, bioactive amino acids have emerged as potential additives for improving hatchery performance due to their physiological and neurochemical roles in larval development. Taurine, one of the most abundant free amino acids in marine molluscs, accumulates during larval development and has been shown to improve larval growth and survival in *Crassostrea gigas* [2]. L-aspartic acid acts as a metabolic precursor of D-aspartate, an endogenous agonist of *N*-methyl-D-aspartate (NMDA) receptors, and NMDA-mediated signalling has been implicated in the regulation of larval development and metamorphosis in bivalves [3]. Therefore, this study evaluated the effects of taurine (TAU) and aspartic acid (ASP), at different concentrations and exposure periods, on the induction of metamorphosis in competent *Ruditapes decussatus* larvae.

Material and Methods

R. decussatus larvae were reared in filtered and sterilised seawater (FSW) and fed daily with a bispecific diet of *Tisochrysis lutea* and *Chaetoceros calcitrans*. Competent larvae (approximately 18 days post-fertilisation), identified after being retained twice on a 150 µm sieve, were exposed to taurine and L-aspartic acid at two concentrations (10^{-5} and 10^{-6} M) for 1, 2 or 3 days. Approximately 1000 larvae were placed in glass beakers containing 300 mL of FSW and a 150 µm mesh sieve, and maintained under continuous aeration at 21 °C and a salinity of 33.2. After each exposure period, larvae were removed from the treatment solution, rinsed with FSW, examined under a microscope and transferred to clean FSW until completing six days of the experiment. Larval developmental stages, including full metamorphosis confirmed by the presence of branchial arches, and mortality were assessed immediately after each exposure period and again on day 6. Differences in metamorphosis and mortality were assessed using the Aligned Rank Transform (ART) procedure, followed by Tukey's post hoc test ($p < 0.05$).

Results and Discussion

The effects of taurine and L-aspartic acid on larval metamorphosis and mortality after six days are shown in Fig.1. An exposure to a concentration of 10^{-6} M of L-aspartic acid resulted in the highest full metamorphosis rate observed, with a 2-day exposure inducing $53.13 \pm 12.36\%$ of metamorphosed larvae. As a metabolic precursor of D-aspartate, an endogenous agonist of NMDA receptors, L-aspartic acid may increase D-aspartate availability, therefore enhancing NMDA-mediated signalling associated with larval development and metamorphosis [3]. Nevertheless, the underlying mechanisms remain to be elucidated. Taurine also promoted metamorphosis, with the highest metamorphosis rate obtained after a 2-day exposure to a concentration of 10^{-6} M ($43.33 \pm 6.67\%$). In contrast, the higher concentration (10^{-5} M) of both amino acids failed to induce metamorphosis, regardless the exposure time, with the most larvae remaining at the foot stage, suggesting that increasing concentration does not enhance metamorphic competence and may exceed the optimal threshold. Interestingly, exposure to taurine, particularly at 10^{-6} M, resulted in the appearance of an additional stage, with larvae simultaneously displaying velum, foot and well-developed branchial arches. This developmental stage may represent a transitional stage in which organ differentiation proceeds before complete loss of the velum, suggesting that taurine modulates the timing and coordination of metamorphic events rather than simply accelerating metamorphosis per se. Alternatively, taurine may enhance the differentiation of branchial structures while delaying velum resorption, resulting in a temporary overlap of larval and juvenile characteristics. Further studies are required to elucidate the mechanisms underlying the effects of these amino acids on bivalve metamorphosis.

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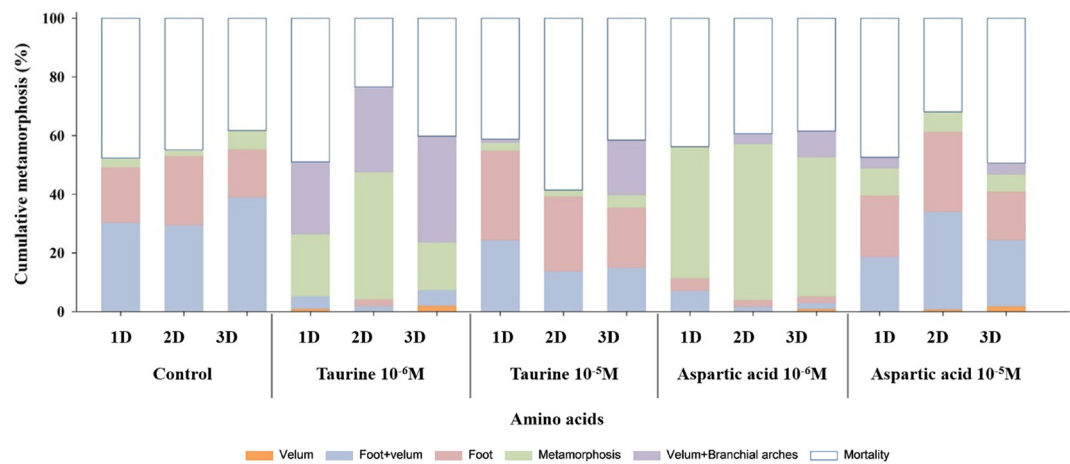


Fig. 1. Larval development and mortality on day 6 following exposure to taurine and L-aspartic acid at two concentrations (10^{-6} and 10^{-5} M) for 1, 2 or 3 days (D).

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TESTING THE WATERS: ACCEPTANCE OF AQUACULTURE FISH AMONG SECONDARY AND UNIVERSITY STUDENTS

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Introduction

In recent years, there has been a growing awareness of the need for sustainable food sources to feed a growing global population while minimizing the environmental impact of agriculture, like aquaculture products. Improving European consumers' perception of these food sources is not only essential for diversifying diets but also for promoting sustainable and environmentally friendly food production. There is an ample body of evidence highlighting the negative perceptions of European consumers towards aquaculture. Studies have shown that even in areas where aquaculture is generally accepted, such as the south of Spain, consumers still believe that wild fish have a greater quality, are healthier, tastier, and more environmentally friendly, even if these opinions are not supported by evidence (Ruiz-Chico et al, 2020). In France, the second largest importer of salmon in Europe, a study has analysed French newspapers and magazines and concluded that most articles about salmon were negative, especially those regarding health (Govaerts, 2021). However, science and fact-based communication campaigns improve the societal perception of aquaculture practices and products (Carrassón et al, 2021), with the amount of available information, as well as the receptor's ability to understand it, being a critical factor to achieve a positive attitude toward aquaculture (Fernández-Polanco and Luna, 2012). To address these issues, it is crucial to educate consumers, providing clear, science-based information about the benefits of aquaculture and insects as foods, which can help dismiss myths and misconceptions, shift perceptions and build consumer confidence in these alternative food sources. This study presents the results of a questionnaire on beliefs and attitudes towards the consumption of aquaculture fish in two groups of young people in Portugal: secondary school and university students. The main objective is to provide empirical evidence to support the design of educational interventions and communication campaigns on sustainable food, in particular with regard to the acceptance of aquaculture products.

Materials and Methods

To map the attitudes and beliefs towards aquaculture products a short online questionnaire was administered to secondary education and university students. Students' ages were coded into categories (1 - 5) and later grouped into three age groups (tertiles). Secondary (highschool) students' gender distribution was 16 girls, 14 boys, 5 "I prefer not to answer" and 8 omissions. University students' gender distribution was 55 women, 32 men and 1 omission. The questionnaire included 19 items about aquaculture fish. Four items were yes/no questions related to consumption patterns and 15 items (e.g., taste preferences, safety, nutritional value, sustainability) were answered on a scale of quotation from 1 to 5, in which higher values indicate more positive attitudes/greater acceptance. We constructed one indicator, **fish_score** with the mean value of these 15 items. Data analysis included descriptive statistics (M - Mean, SD - Standard Deviation) of fish scores by group, and Student's t-tests for independent samples to compare: a) Secondary education vs university, b) Gender (male vs female) at each level of education.

Results and Discussion

In both secondary education (N = 43; M = 2.99, SD = 0.65) and university (N = 88; M = 3.16, SD = 0.41) groups, aquaculture fish is rated moderately positively (mean around or slightly above 3), although slightly more favourable at university. However, Student's t-test (Welch) indicated $t(\approx 66) = -1.44$, $p = 0.157$, with no statistically significant differences ($p < 0.05$) between secondary education and university students opinions. The attitudes of both genders (Table 1) are practically identical, with no gender effect. Student's t-test (Welch) indicated $t(\approx 26) = 0.47$, $p = 0.640$ for secondary education (boys vs girls), and $t(85) = -0.29$, $p = 0.772$ for university (men vs women) students.

These results show a relative normalization of aquaculture fish in the groups studied, with no significant differences between secondary and university, nor between genders, suggesting that resistance to aquaculture fish is not the main obstacle in this young audience, although specific beliefs (e.g. presence of chemicals, use of antibiotics) remain that can be worked on. Based on the results and on specific item averages (results not shown), some guidelines for activity planning can be highlighted: 1) Evidence-focused education on food safety, nutritional quality and sustainability, consolidating an already moderately positive perception and clarifying myths (chemicals, antibiotics, etc.); 2) The data do not suggest the need for radically different interventions for different genders; a common discourse, with minor language adjustments and examples depending on the school/university context, may be more effective.

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Table 1. Means and standard deviations of attitudes towards aquaculture fish by education level and gender.
Note: Scale from 1 to 5; Higher values indicate more positive attitudes.

Group	Gender	N	fish_score M (SD)
Secondary education	Boys	14	3.03 (0.80)
	Girls	16	2.90 (0.61)
University	Men	32	3.14 (0.42)
	Women	55	3.17 (0.41)

Acknowledgment

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INTEGRATION OF HORMONAL PROFILING AND GONADAL HISTOLOGY FOR MATURATION ASSESSMENT IN COMMON CARP *Cyprinus carpio* BROODSTOCK

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Introduction

Common carp (*Cyprinus carpio*) is historically and currently one of the most important farmed finfish species globally, especially across Asia and Europe, due to its adaptability, robust nature, and economic value as an affordable protein source. The reproductive biology of carp is crucial for maintaining productivity in fish farming. Reliable identification of reproductive maturity in common carp broodstock is critical for spawning management, hatchery planning, and improving seed production efficiency (Dadras et al., 2016). Determining fecundity and development of sexual maturity is a fundamental aspect of fisheries science, especially aquaculture. Conventional approaches based solely on external phenotypic assessment often lack precision, particularly outside the peak reproductive season. Gonad development and reproductive rhythm of fish are closely linked to changes in environmental temperature (Li et al., 2024). Female carp can reach sexual maturity at 2-3 years of age, and when the water temperature increases to approximately 18 °C, they begin to lay eggs and reproduce (Hao, 2012). This study aimed to demonstrate that integrated endocrine (sex-steroid and biochemical) profiling provides a reliable, non-lethal confirmation of sexual maturity in common carp broodstock when validated against gonadal histology, thereby establishing hormonal assessment as a robust primary tool for maturation evaluation in this species.

Material and methods

Blood samples (n=15) from broodstock were analyzed for plasma testosterone, estrogen, progesterone, and key biochemical markers (calcium, albumin). Serum sex steroids (testosterone, estradiol and progesterone) were quantified using commercially available electrochemiluminescence immunoassays analyzed on the Roche Cobas e411 platform. Samples were analyzed individually, and results were exported for comparison with histologically defined maturation stages. Hormonal interpretation was based on extracted dataset descriptions, including detection limits and concentration ranges for each hormone. Routine histology was used to prepare tissue sections for histological analysis. After fixation in Davidson fixative, tissues were further dehydrated with increasing alcohol concentrations, cleared in xylene, and soaked in paraffin wax using an automated Excelsior AS processor. Paraffin block sections were cut into 2 µm sections using a Microm HM 355S microtome. Tissue sections were stained with modified Harris hematoxylin and Young's eosin for histopathological evaluation and with periodic acid for visualization of mucosal substance. Photomicrographs were taken with Axio Scan.Z1 scanning light microscope and processed with image processing software ZEN 2.3. Histological maturity classifications for all samples were obtained from the gonad histology report.

Results and discussion

Sex-steroid profiles varied distinctly among broodstock. Several individuals exhibited testosterone concentrations near or below the detection limit (<0.03 ng/mL), indicating non-active or immature gonads, whereas others showed elevated testosterone (0.68–0.82 ng/mL), consistent with mature males. High estrogen levels (291.5–726.2 ng/mL), often coupled with elevated progesterone (0.12–0.37 ng/mL), characterized hormonally mature females. Samples with uniformly low concentrations of all steroids suggest immature or non-reproductive individuals.

The blood biochemistry report additionally highlighted the functional importance of calcium for oocyte development and albumin for egg quality and reproductive performance in females. Multiple histology samples were identified as mature females, several as mature males, and a subset as immature fish; all findings were reported as being fully consistent with hormonal profiles. The combination of endocrine and histological analyses provided clear and mutually reinforcing indicators of sex and reproductive maturity in common carp. Elevated estrogen and progesterone reliably signaled mature females, whereas high testosterone identified mature males. Individuals with suppressed levels of all steroids were confidently classified as immature, which histology fully corroborated. The strong concordance between hormonal and histological data supports endocrine screening as a non-lethal, rapid and cost-effective first-line tool, with histology reserved for confirmation, while calcium and albumin add valuable biochemical context on oocyte development and overall broodstock reproductive performance.

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Conclusions

Combining endocrine profiling with histological validation ensures accurate maturity assessment in common carp. This integrated approach optimizes hatchery selection and spawning, leading to more efficient and sustainable production.

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RESTOCKING STRATEGIES FOR REBUILDING WAR-AFFECTED ICHTHYOFAUNA IN UKRAINIAN WATER BODIES

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Introduction

One of the consequences of the military aggression in Ukraine is a sharp deterioration in the conditions of natural reproduction of fish, in particular due to the disruption of the hydrological regime during the spawning period and, consequently, the disruption of the normal recruitment of the reproductive core of fish populations. In turn, this causes a decrease in biological diversity, as an element of the stability of hydrobiocenoses and a deterioration in the conditions for the provision of environmental services due to the exploitation of the resource base of fishery.

Materials and methods

The work is based on the results of field studies of the Institute of Fisheries of the National Academy of Agrarian Sciences conducted in 2020–21 in the Pechenihy (86.2 km²), Oskil (area of 123.0 km²) and Karlivka (area of 2.5 km²) reservoirs, the purpose of which was to determine the parameters of the ichthyocomplex in the context of the state of the resource base of commercial fishery. The assessment of losses from the disruption of spawning conditions was carried out in accordance with the methodology for assessing losses to the fishery industry in force in Ukraine in a modification of the preliminary determination of fish productivity of spawning grounds based on the abundance and average biological parameters of brood fish of the main commercial species. Hydrological parameters were taken from the official data of the regional offices of the State Agency of Water Resources of Ukraine.

Results

The studies on water bodies located in the combat zone showed a drastic deterioration in spawning conditions in all transformed water systems with a regulated water level regime in the period 2022–24 up to the complete exclusion of the littoral zone from the spawning fund. The military hostilities prevented the normal filling of the Pechenihy reservoir (Kharkiv region) during the spawning period. As of 04 May 2022 (i.e. during the period of mass spawning of the main commercial fish species of the Pechenihy reservoir), the water level was 98.56 m, i.e. 1.94 m below the normal water level. The calculations showed that the losses of the ichthyofauna of the Pechenihy reservoir can be estimated at 740 tons, of which 21.9% was *Abramis brama*; 19.9% - *Carassius gibelio*; 14.8% - *Cyprinus carpio*; 6.8% - *Sander lucioperca*.

As a result of damage to the dam of the Karlivka Reservoir (Donetsk region), the water level as of 31 May 2023 dropped by 3.63 m with an average reservoir depth of 4.2 m. Accordingly, all littoral areas that formed the habitats of reproduction of commercial fish species were drained with a complete loss of fish productivity in spawning grounds in 2023. The total loss was estimated as 33 tons, of which 55.7% was accounted for by *C. carpio*; 37.5% - by *C. gibelio*.

Thus, there was a noticeable decrease in the brood stock of fish under the influence of military actions, which raises the question of ways to restore it in the post-war period. This problem should be solved in several ways, one of which, in our opinion, should be the introduction of juveniles from brood fish of local origin produced in aquaculture. The main paradigm within which artificial reproduction of ichthyofauna should be carried out is an increase in fish productivity, replenishment of reproductive stocks of small populations and the implementation of biological amelioration. Obviously, in water bodies with fishery activities, the first component should prevail, and while the second way should be implemented in other water bodies. Due to artificial reproduction, it is necessary to select a complex of species that provides an optimal trophic load on the main food groups of aquatic organisms, with priority for the introduction of species, the abundance and biomass of which are lower than that typical for a balanced ichthyocenosis. At the same time, in conditions of a sufficiently high level

of human impact, when planning measures for artificial reproduction, it is necessary to take into account the resistance of introduced species to the effects of unfavorable environmental conditions, as well as the availability of a proven technology for obtaining juveniles in sufficient quantities. The following can be defined as basic objects for artificial reproduction of the ichthyofauna: native predators - *Sander lucioperca*, *Esox lucius*, *Silurus glanis* and benthophages – *C. carpio*, *A. brama*, *Tinca tinca*. The native ichthyocomplex is unsuitable for the implementation of biological amelioration, therefore these measures should be focused on alien species such as *Hypophthalmichthys molitrix* and *Ctenopharyngodon idella*.

Currently, Ukraine has accumulated sufficient experience in implementing measures for the targeted formation of ichthyofauna. If in the main inland water bodies of Ukraine - the Dnieper reservoirs, no more than 10% of the commercial catch was ensured due to artificial propagation measures, while in small and medium water bodies it has amounted to 70-90% in recent years.

Given the unfavorable forecast for the dynamics of the reproductive potential of commercial fish species and the high degree of regulation of the flow of rivers that suffered from military actions, natural reproduction will not ensure sufficient efficiency of restoring the fish fauna. Accordingly, the problem of restocking fish populations at the expense of juveniles grown in aquaculture conditions will become urgent. At the same time, in the immediate post-war years, large-scale stocking of fish at the expense of state programs is problematic. A solution may be the transfer of such water bodies for long-term use for the purposes of fishery with the obligatory condition of stocking them with native species. In this case, fishery activities can be carried out with fishing gears with a mesh size of at least 90 mm, which will ensure a minimum load on the brood fish of the majority of native fish species. The mission of the state in this case will be to create regional fish hatcheries that will grow viable fish seeds from species of local origin.

The study 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.

ENVIRONMENTAL, ECONOMIC, AND NUTRITIONAL CHALLENGES TO THE INCLUSION OF INSECT PROTEIN IN EUROPEAN UNION AQUACULTURE

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In the last decade, insectmeal and insect oil have emerged as novel ingredients in aquaculture systems in Europe, bearing the promises of diversifying feed sources, avoiding overfishing, reducing the environmental footprint of aquaculture, and enhancing growth. The European Union (EU) regulatory framework governing aquaculture and insect farming has changed to allow for insect protein to be channelled into aquaculture systems. However, this framework remains incomplete, with several regulatory gaps, and is subject to change as the business model for insect farming is not settled.

The renewal of aquaculture guidelines, and of the Common Fisheries Policy, provides an opportunity to evaluate the potential of insect ingredients in aquaculture and to critically assess their environmental, economic, and nutritional claims. This assessment should inform the development of the regulatory framework for insect farming and aquaculture in the future. Eurogroup for Animals, the federation of animal protection organisations in the European Union, has been reporting on insect farming in the EU, and has identified three crucial limitations that impedes its development as a feed source for aquaculture.

The environmental benefits of substituting insectmeal to fishmeal are not clear cut. Recent analysis found the early sustainability claims of insect proteins to be flawed by an over-reliance on a handful of old and incomplete studies and exaggerated assumptions, while hopeful industry Life Cycle Analyses (LCA) are not publicly available. Comprehensive studies, such as a sectoral LCA commissioned by the British government, find insect protein to have a climate change impact as high as 4.2 times the impact of fishmeal. Even with circular insect production (feeding insects with food waste, which is currently unauthorised), insect meal would have a higher environmental impact than fishmeal.

Second, the economics of insect farming as a fishmeal replacement or as a feed additive fail to materialise. Currently, insect meal is three to four times more expensive than fishmeal and future projections do not see insect protein retailing at a lower cost than what it seeks to replace. What's more, with insect products' protein content is frequently overestimated by about 20%, inclusion of insect feed would therefore lead to costlier and less nutritionally sound feed. Once presented as a long-term market for insect products, aquaculture is now distanced by more profitable markets, notably in pet food nutrition. Several insect industry leaders that banked on the aquaculture market in the first place have filed for bankruptcy despite having raised hundreds of millions of euros in investments.

Third, the nutritional profile of insect meal and oil, their effects on fish health and growth, and their role as vectors of pathogens and contaminants, are poorly understood. The inclusion of insect meal is limited to 25-30% in farmed fish diets, the disparity in amino acid and lipid profiles between the numerous insects species authorised makes feed formulation complex and raises concerns regarding their compatibility with fish health. What's more, the nutritional benefits of insect products are highly dependent on the substrate that was given to the insects, leading to inconsistencies that are bound to increase if waste streams are authorised in insect farming. This would also bring up the risk of heavy metal accumulation and animal disease transmission, an overlooked topic that could well endanger farmed fish health and welfare.

The inclusion of insect protein as a novel feed ingredient in EU aquaculture comes with several challenges that need to be addressed to ensure that they do not increase the environmental impact of the sector, the competitiveness of the EU, and farmed fish health and welfare. Rigorous assessments should be conducted to inform the evolving regulatory framework for insect farming and aquaculture in the EU.

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Acknowledgment

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EXPERIMENTAL AND NUMERICAL HYDRODYNAMIC INVESTIGATION OF AN OFFSHORE LONGLINE AQUACULTURE SYSTEM

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Introduction

Bivalve aquaculture is a sustainable method for producing high-quality protein, requiring no freshwater inputs and relying on naturally available food sources in the water column. Its expansion into offshore environments offers significant potential for increased production, but introduces higher hydrodynamic loads that challenge system integrity and survivability (Buck *et al.*, 2017). Longline systems are the most widely used offshore configuration, consisting of a surface-supported main line with suspended droppers or lantern nets. This study investigates two design variations: a conventional surface system and a modified submerged configuration with reduced surface flotation and deeper line positioning. Based on previous work (Aromatario *et al.*, 2025), the submerged design is expected to experience reduced wave-induced loads. The objective is to evaluate its performance relative to the surface system and to validate a numerical model for simulating a broader range of environmental conditions and designs.

Methods

Two variations of a longline system were experimentally investigated at a 1:12 Froude scale in the Concept Basin at MARIN. Each test section comprised five sets of lantern nets, designed to reproduce full-scale wet weight and hydrodynamic drag. Load sensors measured tensions in the main longline and in representative lantern and buoy lines, while system motions were recorded using a camera and processed to extract trajectories of selected elements.

A numerical model was developed in OrcaFlex using a lumped-mass approach, with lines discretized into segments where mass, buoyancy, and hydrodynamic forces are applied at the nodes. Lantern nets were modeled as line objects with infinite bending stiffness. Surface cylindrical buoys were represented as 6D buoys, and submerged spherical buoys as 3D buoys. Hydrodynamic loading was computed using a Morison-type formulation.

Experimental and numerical results were compared in terms of tension loads and motions. Harmonic analysis was applied to regular waves, and spectral analysis to irregular wave conditions.

Results and Discussion

Results show that the submerged configuration reduces peak loads on the main longline by up to 40%. Motions of the lantern nets are also reduced in both horizontal and vertical directions, consistent with the decay of wave orbital velocities with depth.

The numerical model accurately reproduces system motions, capturing both the shape and extent of orbital trajectories. However, tension predictions are more challenging. The simplified hydrodynamic formulation, based on empirical drag and inertia coefficients, limits the accuracy in representing complex flow interactions. Mean loads are generally overestimated, while wave-frequency components are slightly underestimated, particularly under steeper wave conditions. Calibration of hydrodynamic coefficients improves agreement between numerical and experimental results.

Overall, the results highlight the potential of submerged longline designs to reduce structural loading and improve offshore aquaculture resilience, while also identifying key limitations and calibration needs for numerical modeling approaches.

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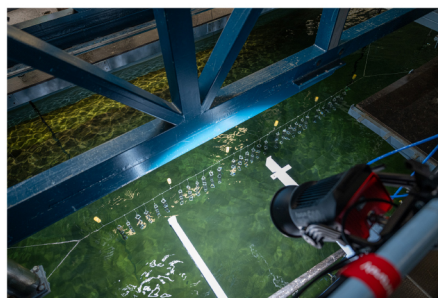


FIGURE 1: Experimental setup in the concept basin at MARIN.

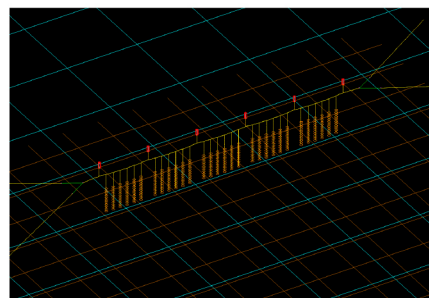


FIGURE 2: OrcaFlex numerical model.

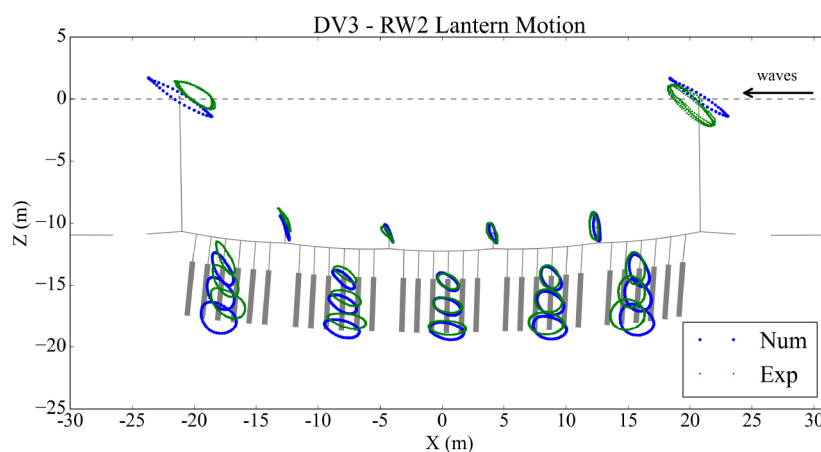


FIGURE 3: Comparison of orbital motions for the submerged design (DV3) under regular wave RW2 ($H=3.45\text{m}$, $T=10.68\text{s}$) for experimental vs numerical results.

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BIOLOGICAL PARAMETERISATION OF SEA LICE MODELLING: A REVIEW OF BIOLOGICAL DATA, KNOWLEDGE GAPS, AND EXPERIMENTAL PRIORITIES

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Introduction

Sea lice remain a huge constraint for the sustainability of the aquaculture industry (Brooker *et al.*, 2018; Moriarty *et al.*, 2024). Their impact has driven the development of management tools, particularly models that can predict population dynamics, simulate dispersal between connected farms and wild populations of fish, and evaluate the efficacy of control strategies (Harrington *et al.*, 2023; Revie *et al.*, 2005). Particle-tracking models coupled with hydrodynamic components can simulate sea lice life cycles and transmission under variable environmental conditions. Sea lice dispersal models have become more readily integrated into management frameworks by determining appropriate treatment, stocking and fallowing periods of specific aquaculture zones (Vollset *et al.*, 2018). However, model application and effectiveness remain inhibited by limited biological data. Knowledge gaps have been identified in previous reviews, highlighting the limitations arising from incomplete or outdated representations of louse biology (Brooker *et al.*, 2018; Moriarty *et al.*, 2024). Key processes influencing the planktonic larval stages require particular attention, as they remain poorly parameterized. We systematically evaluate the range of biological parameter values used in salmon lice dispersal models, with particular emphasis on planktonic larval stages, to distinguish empirically supported parameters from those that are assumed or weakly supported. By examining areas where empirical data are lacking, and assessing the extent to which generalised parameter values are applied across studies, we have identified priority areas where laboratory and field studies could improve parameterizations.

Methodology

To carry out a comprehensive search for papers that describe the development of dynamic sea lice models using biological data, keywords and Boolean logic were applied in Web of Science and Scopus. Articles went through an exclusion process resulting in 81 papers ultimately being assessed for biological parameterisation. Biological sea louse parameters were chosen for their importance to the infection process and modelling outcomes. These included data on ovigerous females, such as their abundance and source of data, as well as key fecundity-related parameters including egg number, egg replacement rate and egg viability. Additional parameters considered were hatching rates, larval development rates, mortality rates and swimming behaviour.

Results and Discussion

We determined that larval development rates are generally well characterised, however there is a reliance on outdated or limited data in other key areas including female reproduction, larval mortality, and larval swimming behaviour. These findings illustrate a need to better incorporate more scientifically rooted biological and environmental variability into models to improve the accuracy of their predictions and in turn strengthen sea lice management. Consequently, experiments aimed at strengthening these knowledge gaps have been developed. We will present preliminary data on the role of predation on influencing numbers of planktonic sea lice larvae including identifying the main planktonic predators. Additionally, experiments looking at how fecundity varies spatially and between lice from both wild and farmed fish has become a greater focus to eliminate uncertainties and will also be discussed.

(Continued on next page)

Acknowledgment

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DEVELOPMENT OF A TOTAL INORGANIC NITROGEN ANALYSER FOR WATER QUALITY MONITORING IN FRESH AND SALINE AQUACULTURE PROCESSES

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Water quality is one of the most critical determinants of fish health, performance, and mortality in aquaculture facilities. Among all water-quality parameters, real-time monitoring of ammonia is particularly important due to its acute toxicity in its unionised (NH_3) form. Ammonia is naturally produced through the nitrogen cycle and exists in equilibrium with its ionised form, ammonium (NH_4^+), depending on pH and temperature. In aquaculture environments, even short-term elevations in unionised ammonia or total ammonia nitrogen (TAN) can disrupt cellular processes, cause stress, suppress feeding, and ultimately lead to mortality. This challenge extends beyond facilities: ammonia released into receiving waters contributes to nutrient imbalance, eutrophication, and wider ecosystem impacts, linking reliable monitoring directly to licensing and environmental compliance.

To date, the measurement of nitrogen species has been difficult for RAS end users, where high costs are met with unreliable data due to the challenges some technologies face from interference and biofouling. Current practice relies heavily on manual colorimetric tests or unstable ion-selective electrode (ISE) probes, both of which suffer from drift, fouling, interference from salinity and pH, and require constant recalibration. These limitations leave operators with low-frequency, low-confidence ammonia data that often misses rapid spikes caused by feeding events, biofilter instability, disease treatments, or equipment failures. Additionally, sporadic analysis does not arm the operators with the granular data required to be in total control of water quality, and the grow out processes.

Researchers at Aquamonitrix have developed a novel methodology for the detection of Ammonia, based on the conversion of ammonia to a UV-chromophore. Ammonia cannot directly be measured by standard UV-Vis spectroscopy (200 nm – 1,100 nm), due to its absorbance lying outside this window*. However, indirect detection (i.e. converting ammonia into another chemical form) however, is a proven, robust method for ammonia detection. This allowed for the development of an analyser that delivers simultaneous detection of ammonia, nitrite, and nitrate. The proposed method, which employs rapid, high-capacity ion chromatography with UV-LED detection, has been tested and validated in the laboratory at Aquamonitrix facilities, through the construction of a prototype analyser, that is able to in-situ convert ammonia into a UV-active chromophore, while simultaneously measuring and quantifying all three primary inorganic nitrogen species. This analyser was demonstrated at a flow-through aquaculture farm where it monitored ammonia, nitrite, and nitrate for a period of weeks. In summary, the work here-in will present a real-time, online total inorganic nitrogen (TIN) analyser with low maintenance requirements (with uptimes of $\geq 85\%$), that is highly accurate ($\geq 90\%$) and precise ($\geq 90\%$), resistance to bio-fouling and can operate in both fresh and saline RAS farms without any user-level actions. The analyser has a low environmental impact and provides real-time data through IoT connectivity.

This technology will facilitate new possibilities for improved control of the aquaculture facilities and will deliver increased resource utilisation efficiency. The real-time monitoring of ammonia, (alongside nitrate and nitrite), provides important information that can be used to implement process controls with regard to feeding. Next to energy, feed is one of the biggest green-house emitters for the aquaculture industry, and with the general trend towards renewable energy, it is increasingly becoming the largest source of environmental burden. Thus, the implementation of this technology, will help to improve the feed-conversion ratios (FCR) of RAS operators, and as a result, reduce their overall environmental burden. Equally, by allowing RAS operators to optimise their stocking densities, the CO_2 cost per fish will be reduced, thereby reducing the overall greenhouse green-house burden of the farm, while also minimising energy consumption across RAS operations, mainly by optimising stock density and reducing water exchange.

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PERFORMANCE OF ATLANTIC SALMON (*Salmo salar*) FED DIETS CONTAINING GENETICALLY MODIFIED CAMELINA OIL ENGINEERED TO RESEMBLE SOUTHERN HEMISPHERE FISH OIL

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Introduction

Reducing aquaculture's reliance on marine resources such as fish oil and fish meal is a key objective for the industry, driven by cost and environmental footprint. However, substitute terrestrial (vegetable) oils lack crucial omega-3 long chain polyunsaturated fatty acids (*n*-3 LC-PUFA), especially eicosapentaenoic and docosahexaenoic acids (EPA and DHA, respectively) resulting in flesh with reduced *n*-3 content. This is undesirable for several reasons, linked to a negative impact on the health of the fish and flesh nutritional quality to humans. Plant biotechnological production platforms, in the form of genetically modified (GM) crops are now emerging as a viable alternative to either marine extraction or expensive algal fermentation (Napier and Betancor, 2023). Rothamsted research has developed several iterations of GM *Camelina sativa* accumulating high levels of EPA+DHA in its oil, and previously demonstrated the utility of these oils as an aquafeed ingredient for Atlantic salmon (*Salmo salar*; Betancor et al., 2015; Betancor et al., 2018). In the current trial a new iteration, in which the GM camelina fatty acid profile has been optimised to contain 20% EPA and DHA combined, was tested in Atlantic salmon for performance and health-promoting potential.

Materials and Methods

Three diets (Control, CO15 and CO30) were formulated with varying levels of fish oil (FO), omega-3 GM Camelina oil (CO) or rapeseed oil (RO) as a proportion of the total oil (Figure 1) and produced by Biomar. The diets were produced by hot extrusion with a blend of fish meal (FM), wheat, soy and crystalline amino acids, and oils were sprayed onto pellets by vacuum coating. Triplicated ambient temperature flowthrough 0.75 m², 1.4 m diameter tanks of 55 post-smolt Atlantic salmon (starting weight 200 g) were fed these diets in excess for a total of 19 weeks until all fish had increased in weight at least 4-fold. Uneaten feed was collected daily to calculate feed intake and feed conversion rate (FCR) whilst biometric data recorded at the start and end of the trial was used to calculate growth and performance indices. At the end of the trial whole fish, Norwegian quality cut (NQC), and samples of liver and intestine were collected to evaluate composition (including fatty acid profile) and gene expression relating to nutrient uptake and health. In addition, whole fish were collected at the start of the trial to assess composition and calculate *n*-3 retention.

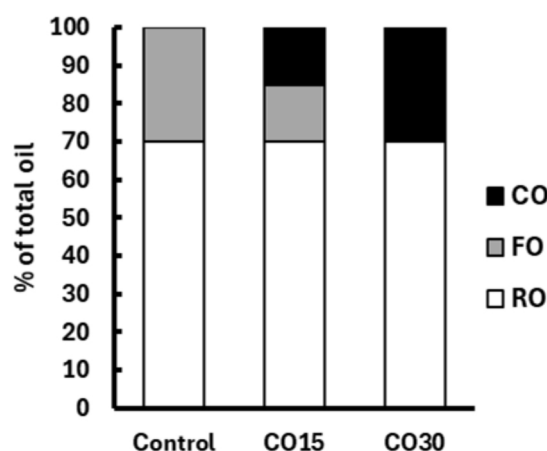


Figure 1. Proportions of rapeseed oil (RO), fish oil (FO) and GM camelina oil (CO) as a percentage of total oil in diets.

(Continued on next page)

Results and Discussion

Weight gain and specific growth rate (SGR) were both higher in fish fed the CO15 diet than those fed the CO30 diet ($P = 0.024$ and $P = 0.016$ respectively). In contrast, FCR was lower in fish fed CO15 diet compared to CO30 ($P = 0.048$), indicating a more efficient uptake and assimilation of this diet. A higher energy content in flesh of fish fed the CO15 compared to those fed CO30 ($P = 0.016$) was complimented with corresponding differences in lipid levels ($P = 0.016$). Fatty acid levels (mirrored the diets fed to specific groups with higher levels of EPA in fish fed CO30 than those fed CO15 ($P = 0.003$) or Control ($P = 0.009$). However, whilst DHA levels were significantly lower in whole fish fed CO30 than other groups the difference in proportions was not as pronounced as in diets, likely due to low levels of biosynthesis as evidenced by the significantly higher retention, at 109%, compared to fish fed CO15 (93%; $P = 0.037$) and Control (77%; $P = 0.044$). Slightly lower growth performance of fish fed the CO30 diet may be related to levels of glucosinolates in the transgenic camelina oil (Betancor et al., 2021). However, better performance and higher absolute levels of total n -3 LCPUFA in fish fed CO15 compared to those fed Control ($P = 0.002$) demonstrate that this iteration of GM camelina oil functions well as part of a blend with fish oil and rapeseed oil in diets for Atlantic salmon.

Acknowledgment

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REVOLUTIONIZING FISH EGG QUALITY ASSESSMENT: RAPID FATTY ACID DETECTION WITH RAMAN SPECTROSCOPY

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Egg quality in aquaculture is often judged by physical traits (egg diameter, symmetry), but biochemical composition, especially n3 fatty acids, more directly predicts developmental potential. DHA, EPA and ARA are linked to higher hatching success and better egg performance. While GCMS provides accurate fattyacid profiles, it is slow, costly and laborintensive.

We evaluated Raman spectroscopy as a rapid, lowcost alternative to estimate DHA and EPA in fish eggs. Raman captures a nearinstant chemical fingerprint from intact samples and is sensitive to unsaturated lipids. We paired Raman spectra with GCMS reference data from 90 spawn samples collected over seven years from California yellowtail (*Seriola* spp.) and Atlantic cod (*Gadus morhua*). After preprocessing, a PLS regression model was trained to predict DHA and EPA from the spectra; model tuning and validation used crossvalidation and leaveseasonout tests to assess generalizability.

The model showed strong performance: $R^2CV = 0.91$ and $RMSECV = 2.5\%$ (see Fig. 1, predicted vs. actual DHA+EPA). Figure 1 also highlights that *Seriola* samples span a wide quality range while cod samples cluster tightly, illustrating model behavior across different sample distributions.

These results demonstrate Raman spectroscopy as a rapid, portable, and costeffective tool for egg quality screening. Deploying Ramanbased monitoring in hatcheries could enable nearrealtime assessment of reproductive performance and help ensure consistent production of robust offspring across multiple species.

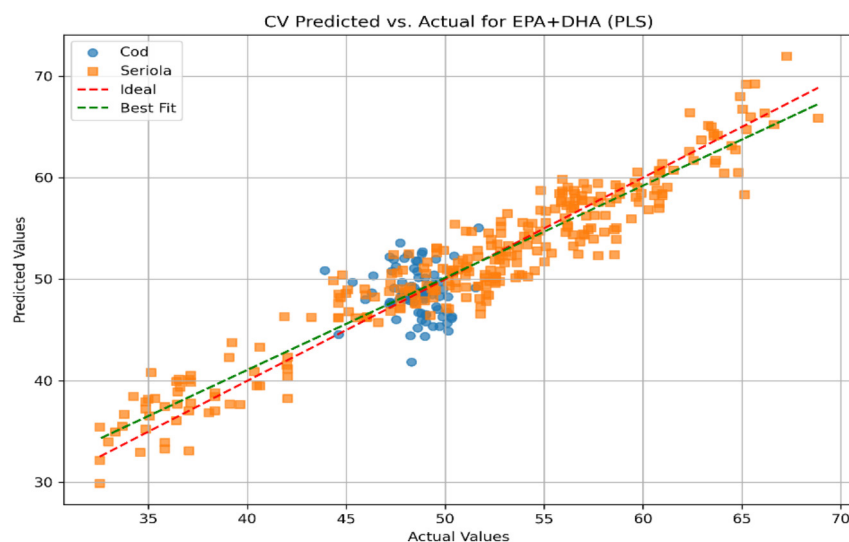


Figure 1: Partial least squares (PLS) regression plot of actual versus predicted EPA+DHA values using Raman spectra in California yellowtail and Atlantic cod eggs

IMPROVING THE PERFORMANCE OF WHITELEG SHRIMP *Litopenaeus vannamei* WITH SINGLE-CELL PROTEIN AND PALATABILITY ENHANCER

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Introduction

Future deficiencies and volatility of the market are pushing feed formulations towards partial replacement of fishmeal by protein rich ingredients, such as vegetable or single-cell proteins. This switch in formulations has been vastly addressed in relation to zootechnical indicators like somatic growth and feed conversion ratio (FCR) in several species (Ruiz et al., 2023; Gisbert et al., 2025). Shrimp depends on the liberation of soluble compounds in the water to start feeding, and diets with lower levels of sea-sourced ingredients generate a lower level of these chemo-sensorial signs. On top of that, different results have been reported about feed consumption with partial replacement of fishmeal with single-cell protein (Biswas et al., 2020; Ruiz et al., 2023). The current experiment tried to elucidate the repercussions of replacing fishmeal in diets with a single-cell protein source (Uniprotein®, Unibio A/S) and a palatability enhancer (Luctamax®, Lucta S.A.) in the performance of whiteleg shrimp (*Litopenaeus vannamei*).

Materials and methods

Five diets were produced: a control with 20 % fishmeal (C), two experimental diets with 10 and 20 % of Uniprotein® (UP10 and UP20, 48 and 96 % fishmeal replacement, respectively) and two more diets supplemented with 0.15 % of Luctamax® (UP10PE and UP20PE, same inclusion levels of Uniprotein® and replacement levels of fishmeal). All diets were formulated to be isoproteic, isolipidic and isoenergetic (SPAROS, Portugal).

Whiteleg shrimp (*Litopenaeus vannamei*) with an average initial body weight (IBW) of 3.94 ± 0.12 g were distributed in 450 L circular tanks (50 shrimp/tank, five replicates per treatment) in RIASEARCH (Portugal). They were hand fed five times per day during 36 days.

Results and discussion

UP10 and UP10PE improved final body weight (FBW) and specific growth rate (SGR) over C diet. Contrarily, UP20 and UP20PE reduced growth significantly. There was a significant effect of Luctamax®, showing an improvement in the Uniprotein®:Luctamax® interaction for FBW and SGR in UP20PE when compared to UP20. FCR increased significantly in both diets with 20 % SCP. Shrimp fed with UP10 and UP10PE showed a higher feed intake (FI) than with C, while it decreased in UP20. FI was compensated by Luctamax®, as shown by UP20PE diet.

Table 1. Growth of whiteleg shrimp (*Litopenaeus vannamei*) fed experimental diets

	CTRL	UP10	UP10PE	UP20	UP20PE	p UNI	p LUC	UNIxLUC
IBW (g)	3.94 ± 0.03	3.95 ± 0.03	3.94 ± 0.03	3.95 ± 0.06	3.94 ± 0.06	0.954	0.722	0.968
FBW (g)	14.84 ± 0.46 ^{b, x}	16.55 ± 0.33 ^{c, x}	16.60 ± 0.25 ^{c, y}	13.01 ± 0.44 ^{a, x}	13.99 ± 0.41 ^{a, y}	<0.001	0.027	0.018
SGR (%/day)	3.68 ± 0.09 ^{b, x}	3.98 ± 0.06 ^{c, x}	3.99 ± 0.06 ^{c, y}	3.31 ± 0.12 ^{a, x}	3.52 ± 0.10 ^{a, y}	<0.001	0.027	0.021
FCR	1.27 ± 0.03 ^a	1.25 ± 0.05 ^a	1.23 ± 0.02 ^a	1.33 ± 0.02 ^b	1.34 ± 0.02 ^b	<0.001	0.978	0.678
FI (% BW/day)	4.03 ± 0.04 ^{a, x}	4.20 ± 0.19 ^{b, x}	4.17 ± 0.06 ^{b, y}	3.86 ± 0.05 ^{a, x}	4.11 ± 0.06 ^{a, y}	<0.001	0.028	0.003

Values expressed as mean ± standard deviation (n = 5). a, b, c superscripts show statistical differences (p < 0.05) for Uniprotein®. x, y superscripts show statistical differences (p < 0.05) for Luctamax®. IBW: initial body weight; FBW: final body weight; SGR: specific growth rate; FCR: feed conversion ratio; FI: feed intake.

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The higher growth and FI of UP10 highlights a possible synergy between fishmeal and Uniprotein®. On the contrary, its increase to 20 % suggests that higher levels can limit the acceptability of the feed. Palatability of single-cell protein has been discussed (Sharif et al., 2021; Glencross et al., 2023). It is well known that bacteria contain cell membrane components and nucleic acids. Nucleic acids can act as palatability enhancers and growth promoters, which could explain the good acceptance of the feed and the growth results from UP10. UP20 shows that either the maximum tolerable threshold of inclusion for single-cell protein, either the minimum level of fishmeal in the diet, could have been surpassed. In UP20PE, Luctamax® allowed to restore the FI up to a level comparable to C diet, which suggests support to the chemo-sensorial signals of the feed.

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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 ($\phi' L = 2.93$) and scientific information related to growth parameters is one of the fundamental factors for population management in an aquatic area.

STRESS RESPIRATION AND CHANGES IN CO₂ CONCENTRATIONS AFFECT THE RISK FOR H₂S TOXICITY – INSIGHTS FROM MODEL CALCULATIONS

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Carbon dioxide (CO₂), alkalinity, and pH are closely linked, but little information exists on how CO₂ fluctuations influence hydrogen sulfide (H₂S) toxicity in aquaculture systems. As fish respire, CO₂ accumulates and lowers pH, which increases the proportion of total sulfide present as toxic H₂S. During periods of elevated activity or stress, CO₂ production rises further, potentially worsening this effect. We calculated how respiration driven CO₂ increases can affect pH and the sulfide balance under typical post smolt RAS conditions (12 °C, 12 ppt, 25–200 mg/L alkalinity as CaCO₃). Our results show that relying solely on inlet measurements can underestimate H₂S risks. At high alkalinity and low CO₂, only 5–10% of total sulfide appears as H₂S at the inlet. If CO₂ increases from 5 mg/L at the inlet to 15 mg/L in the tank, the resulting pH drop could increase H₂S up to 2.4-fold. During stress, CO₂ could reach 30 mg/L, leading to a further ~50% increase in H₂S in the tank. Although higher alkalinity generally reduces overall H₂S toxicity, it also makes systems more sensitive to rapid shifts in the sulfide equilibrium when CO₂ rises. Our results clearly suggest that stress and its secondary effects on pH and chemical equilibria may be the missing link to explain the sudden onset and severity of H₂S mortality events. Risk assessments for H₂S must therefore consider the effects of alkalinity, stress and CO₂ on sulfide balances within a system.

Highlights

(1) Increases in CO₂ raise the risk of H₂S toxicity in aquaculture systems. **(2)** Stress respiration exacerbates H₂S toxicity, creating a potential feedback loop. **(3)** High alkalinity protects from H₂S but also masks risks for toxicity. **(4)** Monitoring H₂S before the fish tanks is not sufficient to assess risks in RAS. **(5)** Risk assessment must consider total sulfide and expected maximal changes in pH.

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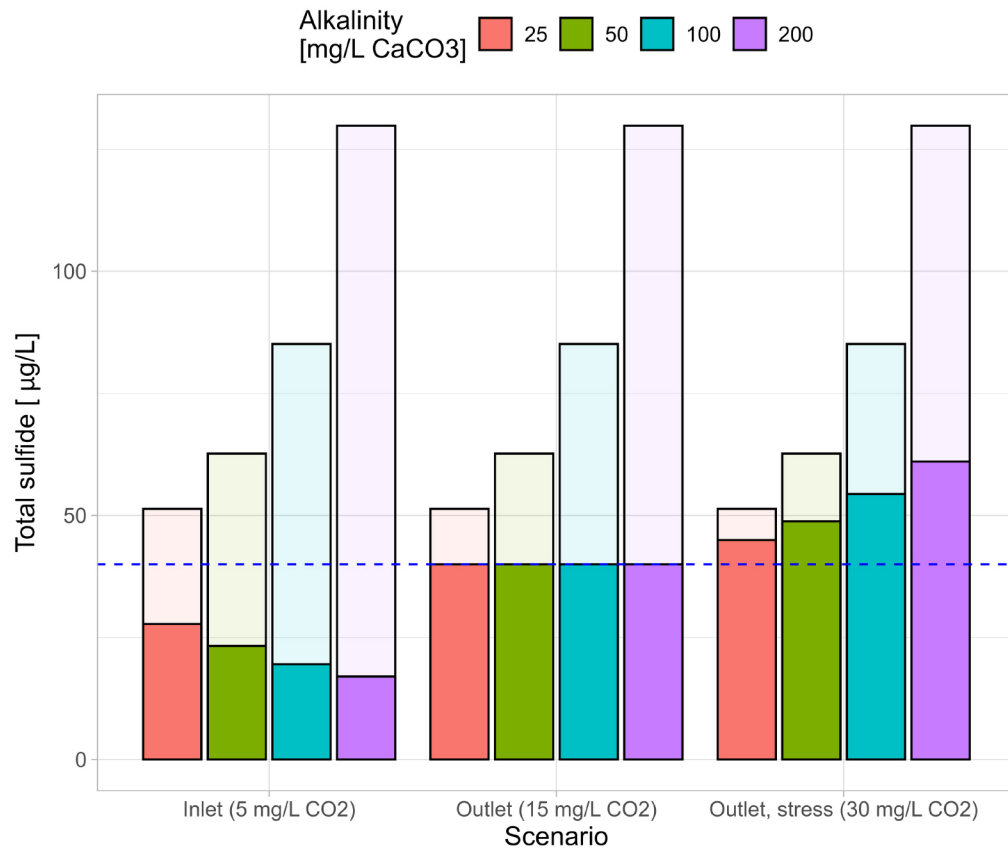


Figure 1: H₂S as fraction of total sulfides calculated for different alkalinities, at tank inlet, at baseline respiration and after stress respiration has been triggered (5, 15 and 30 mg/L CO₂, respectively). The horizontal dashed line indicates the critical limit of 40 µg S/L which could trigger a stress response in a tank stocked with Atlantic salmon (Ciani et al., 2023). The solid part of the bars represents the fraction of total sulfides present as H₂S, total bar height indicated total sulfides (H₂S and HS⁻). (T = 12°C, salinity = 12 ppt).

CHANNELLING SCIENCE TO INDUSTRY

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Introduction

Bridging the gap between science and industry is critical in Asia Pacific, given the wide range of educational backgrounds—from hands-on learners without formal training to those with advanced degrees and professional expertise.

In a competitive aquaculture landscape, particularly in shrimp farming, knowledge, adoption of technology and innovation is the way forward, but in Asia, there has been little open sharing of experiences amongst stakeholders. How can we as media, inspire progress, elevate professionalism, and strengthen the industry's long-term resilience?

Aqua Culture Asia Pacific magazine (AAP) aims to level the playing field by serving as a bimonthly trade publication that provides actionable knowledge and technology. For 22 years, AAP's mission has been to catalyse the industry, acting as a window to the world for Asia Pacific producers, and a door to the market for global suppliers. Through knowledge exchange and adoption of innovation, AAP strives to be a beacon for the industry. This reflective account explores experiences in navigating and bridging the gap between science and industry.

Bridging the science–industry divide

Many scientists struggle to translate their research into tangible benefits for industry. Furthermore, traditional research papers are written in formats that bore or confuse industry professionals. AAP's role is to extract this science, make it attractive, and show industry players how they can profit from it. This presentation will provide examples of how to match science to industry. The aim is to show the scientific community how research can benefit industry and not remain locked in academic journals which the evolving aquaculture industry does not consult. The channeling of science must be a two-way street.

When objectives clash

Industry and researchers have diverse objectives, and researchers often hurry to patent discoveries, leading to concealed knowledge that can hinder industry advancement. This already adverse trend is further exacerbated when researchers try to embark into marketing.

Startup–industry gap

We depend on startups to innovate but here we need to bridge another divide. Many startups are outsiders to the aquaculture world. They focus too much on product features, while failing to articulate the benefits to the industry, and consequently blaming that industry is 'blind'. AAP works to bridge this divide by matching features to benefits and clarifying what the industry truly needs especially in terms of financial returns. This presentation will give examples from articles in AAP's recent volumes.

EFFICACY OF AN INACTIVATED *Lactococcus petauri* VACCINE AGAINST MEDITERRANEAN ISOLATES FROM EUROPEAN SEABASS (*Dicentrarchus labrax*)

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Introduction

Lactococcus garvieae (LG) is an emerging bacterial pathogen of European seabass (*Dicentrarchus labrax*), causing significant economic losses in Mediterranean aquaculture. Recent outbreaks have been reported in several producing countries, including Spain and Italy. Previous studies demonstrated that an inactivated vaccine licensed for trout, provides significant protection against a Spanish seabass isolate of LG [1]. The objective of the present study was to evaluate the protective efficacy of the same vaccine against genetically and geographically distinct LG isolates obtained from seabass outbreaks in the Mediterranean region.

Materials and Methods

European seabass fingerlings (15 g) were randomly allocated into 3 vaccinated and 3 placebo groups (n= 55 each). Fish received a single intraperitoneal injection of either ICTHIOVAC® LG (HIPRA, Spain) or phosphate-buffered saline (PBS). Twenty-eight days post-vaccination, separate experimental challenges were performed using three LG isolates recovered from disease outbreaks produced in different seabass aquaculture farms: one previously characterized Spanish isolate (Atlantic origin) and two recent Italian isolates recovered from the Mediterranean area. Challenges were conducted by intraperitoneal injection following anaesthesia. Mortality was monitored daily for 21 days post-challenge. Vaccine efficacy was assessed by comparing cumulative mortality, Relative Percent Survival (RPS), and Kaplan–Meier survival curves between groups. Statistical analyses were performed using the Gehan-Breslow-Wilcoxon test.

Results

All placebo groups developed severe disease following experimental infection, confirming the pathogenicity of the three isolates in seabass. In all challenge studies, cumulative mortality in placebo fish exceeded 60%, demonstrating the robustness and validity of the experimental infection models.

Vaccinated fish showed significantly reduced mortality in all challenge models. Relative Percent Survival values of 52.38%, 88.89%, and 86.67% were obtained against the Spanish isolate, Italian isolate 1, and Italian isolate 2, respectively at 13 days post challenge.

Discussion and Conclusions

The results confirm and extend previous evidence of protection conferred by ICTHIOVAC® LG in European seabass [1]. Importantly, the vaccine was effective not only against the previously evaluated Spanish isolate but also against two recent Mediterranean isolates recovered from clinical outbreaks in Italy.

The observed cross-protection suggests a substantial degree of antigenic conservation among contemporary LG strains affecting seabass and supports the use of this vaccine as a practical preventive tool in Mediterranean aquaculture. These findings provide further evidence that the vaccine originally developed for trout can contribute to reducing the impact of lactococcosis in seabass production systems.

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OPEN WATER CULTIVATION OF THE PSEUDO-KELP *Saccorhiza polyschides* IN SCOTLAND – AN ADDED VALUE CROP TO SAFEGUARD SEAWEED FARMING

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Open water seaweed cultivation in the North Atlantic is currently dominated by well-established kelp species, *Saccharina latissima* (sugar kelp) and *Alaria esculenta* (winged kelp). Other fast-growing species have received comparatively little attention, including the pseudo-kelp *Saccorhiza polyschides* (furbelows), a high-yielding annual species native to the coastlines of western Europe that has been harvested and utilised by coastal communities for centuries. Despite its rich and diverse compositional profile and substantial application potential, commercial cultivation of *S. polyschides* remains limited to small research-scale sites in Portugal and lacking entirely from the northern extent of the species' distribution.

Here we present findings from a two-year, high-resolution monitoring study performed at an experimental seaweed farm operated by the Scottish Association for Marine Science (SAMS) on the west coast of Scotland. Across two consecutive cultivation cycles (October–June 2023/24 and 2024/25), *S. polyschides* progression in growth, biomass yield, biochemical (% dry weight dwt of ash, total lipid, protein, and carbohydrate content; alginate; antioxidant activity), and elemental composition (%dwt C and N; trace metals) were followed, alongside continuous monitoring of key environmental cultivation conditions.

Growth dynamics and yield were similar across both cultivation cycles, with *S. polyschides* exhibiting exponential growth from mid-February to early June. Maximum weekly growth rates reached 2.3 kg wet weight per meter of growing line (kg wwt/m), corresponding to harvestable yields of up to 30±6 kg wwt/m. Biochemical and elemental composition varied throughout and across cultivation cycles as well as among tissue types i.e. frond, stipe, and holdfast; the latter of which are commonly excluded from processing. Total lipid and protein contents were highest in fronds, ranging from 3.5–5.9 and 4.4–12.2 %dwt, respectively. Other compounds however were significantly higher in stipes and holdfasts compared to fronds, with ash and alginate contents ranging from 56.5–71.8 %dwt and 18.0–23.9 %dwt in stipes, and 45.1–61.7 %dwt and 20.6–23.9 %dwt in holdfasts, respectively.

Seawater temperatures during Spring of the second cultivation cycle were significantly warmer than in previous years and included several marine heatwave (MHW) events. These conditions coincided with a delayed onset of the phytoplankton bloom and influenced the timing, extent, and composition of the biofouling communities associated with the growing seaweed.

Overall, our results demonstrate substantial achievable yields and favourable biochemical profiles, highlighting *S. polyschides* as a promising candidate for commercial cultivation and a range of applications such as food and feed, biostimulants and -fertilizer, and nutraceuticals. It offers a potential alternative to commonly farmed kelp species that may be increasingly vulnerable to ocean warming due to their narrow thermal tolerance. In addition, biomass that is currently undervalued or discarded (stipes and holdfasts) were shown to be rich in compounds of interest for the seaweed food and biostimulant markets. This study provides new insights into the cultivation potential of *S. polyschides* in cooler waters and identifies key lessons to further optimise production methods.

Acknowledgements

This study was funded under the EU H2020 project 'All Atlantic Ocean, Sustainable, Profitable and Resilient Aquaculture' [ASTRAL, grant ID: 863034; Sep 2020–24] and the NSF-UKRI/BBSRC funded International Bioeconomy & Macroalgae Center [IBMC; Jan 2025–Dec 2029].

CROSS-BORDER, TRANSDISCIPLINARY EFFORTS UNCOVER NON-NATIVE AND PEST SPECIES IN ADRIATIC MOLLUSC AQUACULTURE

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Introduction

Aquaculture, particularly mollusc farming, is a major pathway for the introduction and spread of non-native species (NNS) in the Mediterranean Sea (Katsanevakis et al., 2014). Numerous NNS are mollusc pests and parasites that can negatively affect aquaculture production (Mikac et al., 2025). The accidental transport of these hitchhiking species is facilitated by transfer of adult and juvenile molluscs among farms. Tracking their presence and spread remains challenging since many are cryptic species and require molecular analyses for reliable identification. While traditional monitoring approaches face several limitations, innovative methods have recently emerged that leverage the knowledge of local communities (Azzurro, 2018). Limited information on the occurrence of NNS in aquaculture systems, together with insufficient legislative measures at both national and European levels, hampers the development of effective management strategies to contain their spread across the Mediterranean basin. Here, we present the results of a joint Italian-Croatian-Slovenian survey on non-native fauna associated with mussel and oyster farms in the Adriatic Sea, combining morphological and molecular analyses with farmers Local Ecological Knowledge (LEK).

Materials and Methods

Samples were collected in 2024 and 2025 from 11 mussel and 8 Pacific oyster farms along the Italian coast, 5 European oyster farms along the Croatian and one Pacific oyster farm along the Slovenian coast of the Adriatic Sea. Macrofaunal organisms fouling mussels and oysters were identified through morphological analyses, while molecular identification using COI, 16S, 18S and 28S markers was performed on selected species of interest. In parallel, farmers' ecological knowledge was explored through a semi-structured questionnaire administered at 24 Italian mussel farms and 11 oyster farms. Questions addressed farmers' perceptions, observations, and perceived impacts of non-native and pest species, as well as their practices related to translocation of juvenile and adult molluscs.

Results

A total of 22 non-native and 12 pest species were identified in Italian mollusc farms, several of which fall into both categories. Predatory flatworms were prevalent in mussel farms, while distinct non-native *Polydora* worm species were found boring into oyster shells along the three surveyed coasts. *Polydora caeca* and *P. websteri* were reported for the first time in the Mediterranean. Farmers' observations supported these findings, reporting increases in flatworms, ascidians, barnacles, hydrozoans and *Polydora* polychaetes, which they perceive as negatively affecting molluscs and reducing their marketability. While most farmers were aware of NNS and reported their presence, only a few were able to accurately identify them. Farmers also reported translocation practices involving both seed and adult mollusks between Italy, Croatia, Greece, France and Spain, both within and beyond the Mediterranean basin.

Discussion

The high number of NNS recorded in the Adriatic mollusc farms confirms the central role of aquaculture in their spread. Several species are recorded for the first time in association with farmed mussels and oysters in the Mediterranean highlighting that these environments remain understudied and require further research efforts (Mikac et al., in revision). The first record of *P. websteri* and *P. caeca* in the Mediterranean suggests an ongoing expansion of their distribution ranges; however, their presence may have previously been overlooked due to misidentifications (Mikac et al., 2025). In

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this context, molecular approach proves to be essential for accurate species identification and should be implemented in future studies. Farmers' knowledge proved valuable in detecting the presence of NNS and pests and in understanding their introduction pathways (Fossi et al., 2026). However, targeted educational programs are needed to improve farmers' taxonomic skills and to promote active reporting. The frequent transfer of molluscs between aquaculture facilities, both within the Mediterranean and with Atlantic farms, likely contributes to the spread of non-native and pest species in the region. These findings highlight the need for continuous monitoring of NNS in mollusc aquaculture, stronger cross-border cooperation, closer collaboration among farmers, researchers, and authorities, and the implementation of transparent traceability systems to better manage the movement of molluscs and limit the spread of non-native species.

Acknowledgment

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POLICY BARRIERS AND ENABLERS FOR CLIMATE CHANGE ADAPTATION AND MITIGATION IN AQUACULTURE

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The impacts of climate change is already experienced for several types of aquaculture in many regions of Europe, and is expected to become more severe in the years to come. Aquaculture is also meeting increasing demands to contribute to the mitigation of greenhouse gas (GHG) emissions. The European aquaculture industry is working hard, together with research institutions and others, to develop practical solutions that enable adaptation to the multiple effects of climate change and to mitigate emissions.

The actual implementation and use of these solutions may be limited by regulations and policies at both EU-level, as well as nationally or even at regional level within countries. However, policies may also support the development and use of solutions, by e.g. creating or aligning incentives.

We will present an analysis of identified policy barriers and enablers for the practical adaptation and mitigation solutions being developed, tested and implemented in the OCCAM EU-project (<https://occamproject.eu/>). These barriers and enablers have been identified from an analysis of the results from a review of literature, from interviews and discussions with solution developers and aquaculture industry, and from studying policy and regulatory documents.

The OCCAM project have nine different case studies around Europe, where 12 different practical solutions for adaptation and mitigation are being developed (Figure 1). The case studies include low-trophic and higher trophic species, sea-based and different types of land-based aquaculture, equipment, and different types of solutions.

The preliminary results of the study (at the time of writing the abstract, April 2026) point towards some barriers to implementing solutions being within aquaculture policies, and some barriers being in other policy areas, for example in how fish sludge is classified and its use as fertilizer or soil improvement is regulated. The study also discuss and give recommendations for how the solutions may be adjusted to better facilitate wide implementations, and for changes in policies and regulations.

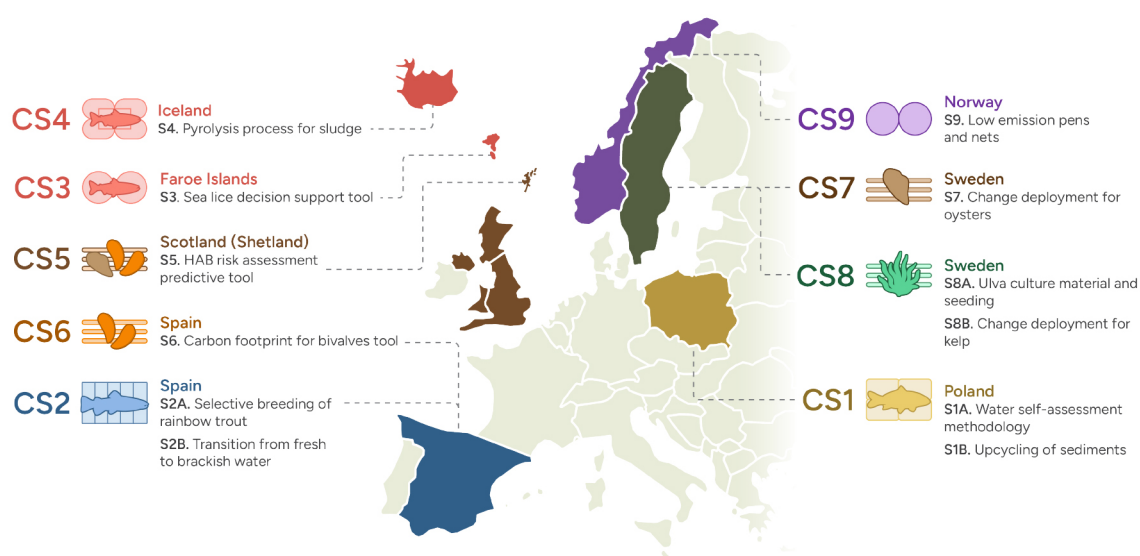


Figure 1: The OCCAM case studies and practical solutions

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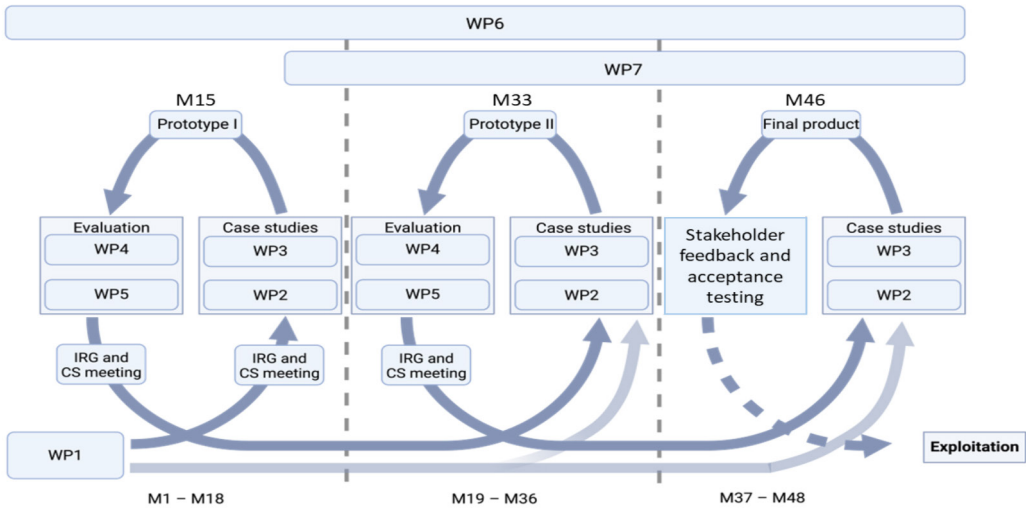


Figure 2: The OCCAM project design with Prototype I and II development loops
Acknowledgment

The OCCAM project and the solutions are currently in the "Prototype I"-loop (Figure 2). In the "Prototype II"-loop, the solutions will be further developed and refined, based on input from this study, as well as testing and evaluation, including in other OCCAM case studies than where they are primarily being developed, and among members of the project's Industry Reference Group.

This study would not have been possible without the information and contributions from other OCCAM project participants.

LASER EXPOSURE AS A MEASURE AGAINST THE PARASITIC SALMON LICE (*Lepeophtheirus salmonis*): EFFECTS ON EXTERNAL AND INTERNAL TISSUE

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In Atlantic salmon (*Salmo salar*) aquaculture, the ectoparasitic salmon louse (*Lepeophtheirus salmonis*) has been one of the main challenges for decades, and a range of measures have been made to prevent and treat infestation. One of these measures is “optical delousing”, which identifies the louse on the salmon and targets laser pulses at it, aiming to kill, injure or detach the louse. However, exact effects of the laser exposure on the lice have not yet been described. The current study investigates survival and potential tissue damage of salmon lice after laser exposure.

Salmon lice were collected after controlled laboratory exposure trials as well as from sea cages equipped with commercial laser systems. Visible damage (pigmentation changes) and survival were assessed, and internal and external changes were investigated by histological analysis and scanning electron microscopy (SEM), respectively.

In laboratory trials, survival varied from low to no difference from control, depending on experimental setup. Pigmentation change increased from day 0 (the day of exposure) to day 1, indicating that laser exposure induces pigmentation changes that develop further after exposure. However, visible damage, measured as proportion of total body surface area with visible pigmentation changes, was not a significant predictor of survival for laboratory exposed salmon lice, nor for salmon lice collected from sea cages with commercial laser systems. Results from SEM revealed effects on the cuticle surface, including regions with uneven and disrupted cuticle structure, but only for some of the regions with visible pigmentation change. Histology only showed minor tissue disruption in oviduct organisation, and no other changes were observed compared to control. Salmon lice from sea cages had disrupted cuticle structure in areas with pigmentation change, and tissue necrosis was observed in histology images. The results indicate changes to pigmentation and external and internal tissue following laser exposure, but the current study could not consistently link these effects to each other or survival.

FROM HISTORICAL OUTBREAKS TO MODERN INTERVENTIONS: CHRONICLING THE IMPACT OF *Vibrio harveyi* AND ESTABLISHING A STANDARDIZED INFECTION MODEL IN BARRAMUNDI

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Acute vibriosis, driven by the halophilic bacterium *Vibrio harveyi*, represents one of the most critical microbiological threats to the expansion of global barramundi (*Lates calcarifer*) aquaculture. Characterized by severe hemorrhagic septicemia, deep dermal ulcerations, and rapid mortality, *V. harveyi* outbreaks frequently devastate nursery and grow-out operations. To develop effective prophylactic interventions, functional feeds, and therapeutic strategies, the establishment of a reproducible, robust, and standardized laboratory infection model is fundamentally required.

The experimental immersion challenge was conducted at the ShrimpVet laboratory facilities using a hyper-virulent field strain of *Vibrio harveyi*. To initiate the challenge, the calibrated bacterial inoculum was introduced directly into the water column of the designated trial tanks. The precise exposure dosage was systematically calculated using ShrimpVet's internal calibration matrices, factoring in both the mean biomass and the specific weight of the fish cohorts at the time of challenge.

Following exposure, real-time mortality trends and clinical signs were monitored at high-frequency intervals over post-infection monitoring period. Moribund and deceased individuals underwent immediate necropsy and bacteriological re-isolation to confirm Koch's postulates, establishing a robust, standardized benchmarking framework for downstream therapeutic screening.

Establishing such predictable mortality baseline minimizes experimental variability across treatment cohorts, ensuring high statistical reproducibility. It offers a valuable platform not only for validating natural phytochemical compounds—such as specialized organic acid formulations—but also for optimizing commercial bath immersion and injection-based vaccination schedules. Ultimately, this standardized protocol bridges the gap between controlled laboratory environments and commercial field conditions, advancing sustainable disease management strategies against vibriosis in global barramundi production.

Acknowledgment

The authors express their sincere gratitude to the experts and technicians at the ShrimpVet laboratory for their invaluable technical support, precise execution of the experimental protocols, and dedication.

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DAILY SCHEDULE FOR BLUEFIN TUNA (*Thunnus thynnus*) SPAWNING AT LAND-BASED FACILITIES

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Introduction

The Atlantic bluefin tuna (*Thunnus thynnus*) is an iconic species that has sustained human populations in the Mediterranean for millennia. Two decades ago, intensive fishing led to severe overfishing of wild stocks, jeopardising the sustainability of the fishery. Although the Recovery Plan established by ICCAT in 2006 has succeeded in restoring these stocks, only production using full aquaculture techniques can guarantee that the increasing demand for this species will be met. To this objective, it is essential to develop full-cycle farming techniques, the first of which is to control its reproduction and the supply of fertilised eggs.

The Spanish Institute of Oceanography (IEO-CSIC) in Cartagena, Murcia (Spain) has the unique facility for the land-based control of Atlantic bluefin tuna (*Thunnus thynnus*) reproduction (www.icar.ieo.es). Since 2023, the tuna has been laying fertilised eggs every year during July and August.

Material and methods

16 adult bluefin tuna fish ranging of 250 kg were housed in a circular tank (22 m diameter, 10 m depth). The fish have grown up in the same facilities over nine years. Fish were fed, at apparent satiety, from Monday to Saturday, once a day with defrost small pelagic fish (mackerel, sardinella, herring, squid...). From 01/01/2026 the photoperiod was the natural one, advanced one month and maintained before 21st may, at 15L:09D. The temperature ranged from 16°C to 26°C, 19.5°C in average. On 3rd July 2026, tunas were implanted underwater with 18 milligrams of GnRH α each, using the technique described by Mylonas *et al.* (2007).

To estimate the time of spawning, the algorithms described by Ortega *et al.* (2024) were used; these enable to estimate the time of spawning based on the stage of embryonic development of the egg and the water temperature, which ranged from 23.7 and 24.8 °C.

Results

Between 6th and 16th July 2026 (both dates inclusive), a total of 20 spawning events with more than 10 million fertilised bluefin tuna eggs were collected in the mornings and afternoons. These eggs were at different stages of embryonic development and, in most cases, exhibited two or more clearly distinct stages, indicating that spawning took place at different times throughout the day. (Figure 1).

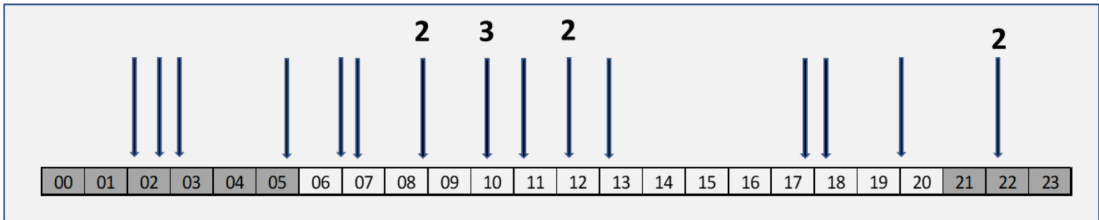


Figure 1. Spawning events estimated (arrows) distributed throughout the 24 hours of the day. The numbers show the spawning events during that same hour when there was more than one. The shading on the hours indicates the hours of daylight and darkness

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Discussion and conclusions

Gordoa & Carreras (2014) confirmed, over four breeding seasons in the Balearic Islands' spawning grounds, that spawning occurred exclusively between 02:00 and 05:00, demonstrating very precise daily synchronization. These results are consistent with De la Gándara *et al.* (2011) observations, which indicate that bluefin tuna kept in floating cages spawn at night and with our own observations from the annual egg collection we carry out in the bluefin tuna fattening cages operated by the company Ricardo Fuentes e Hijos off the coast of Cartagena (unpublished data). In the case of Pacific bluefin tuna (*Thunnus orientalis*) in land-based facilities, Higuchi *et al.* (2023) exhibit a highly synchronized diel spawning rhythm, releasing eggs during the early night, typically around dusk to 22:00 h. But Nyuji *et al.* (2026), pointed that spawning activity of the broodstock can extend from 19:00 to 04:00. In other species of the genus *Thunnus* (*T. albacares*, *T. maccoyii*) kept in land-based facilities, spawning events takes place in the late afternoon, evening and early morning (Benetti *et al.* 2026).

A subsequent genetic analysis of the eggs could indicate which females were involved in each spawning event and whether certain females show a preference for a particular time of day for spawning.

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COMPARISON OF REDOX BIOMARKERS IN PLASMA AND SKIN MUCUS OF ATLANTIC BLUEFIN TUNA

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Introduction

Atlantic bluefin tuna (*Thunnus thynnus*) is a pelagic species with high swimming activity, in which maintaining the balance between oxidants and antioxidants is relevant for preserving cellular integrity and physiological function. In fish, skin mucus constitutes one of the first lines of defence against the marine environment and is continuously exposed to oxygen, microorganisms, and other potentially oxidising agents. Plasma, in turn, provides information on systemic processes in the organism. The joint analysis of both matrices makes it possible to assess whether they provide similar or complementary information on the oxidant-antioxidant balance. The aim of this study was to compare the concentrations and associations of five redox biomarkers in plasma and skin mucus from wild adult *T. thynnus* specimens.

Materials and methods

Plasma and skin mucus samples were collected from 16 adult *T. thynnus* specimens caught in the Barbate tuna trap (Cádiz, Spain). In both matrices, five redox biomarkers were determined: cupric ion reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP), Trolox equivalent antioxidant capacity (TEACH), total thiols, and advanced oxidation protein products (AOPP). Plasma and mucus concentrations were compared using Student's *t*-test. Correlations between matrices, among biomarkers within each matrix, and between their concentrations and body weight were also evaluated.

Results and discussion

All biomarkers showed significantly higher concentrations in skin mucus than in plasma (Table 1). Differences ranged from 1.9- to 3.5-fold for CUPRAC, FRAP, TEACH, and thiols, whereas the AOPP concentration was 21.2-fold higher in mucus. The simultaneous increase in antioxidant capacity indicators and AOPP, as a marker of protein oxidation, suggests that mucus exhibits more intense local redox activity than plasma.

Table 1. Redox biomarkers in plasma and skin mucus from wild adult Atlantic bluefin tuna (*Thunnus thynnus*).

Biomarker	Plasma	Skin mucus	Mucus/plasma ratio
CUPRAC mmol L ⁻¹	0.55 ± 0.20	1.66 ± 0.92	3.0
FRAP mmol L ⁻¹	1.42 ± 0.69	2.67 ± 1.71	1.9
TEACH mmol L ⁻¹	0.56 ± 0.18	1.96 ± 1.22	3.5
Tiol µmol L ⁻¹	136.06 ± 30.90	391.40 ± 154.96	2.9
AOPP µmol L ⁻¹	155.57 ± 46.72	3297.00 ± 1644.62	21.2

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No significant correlations between plasma and mucus were detected for any biomarker. Within each matrix, seven of the ten possible correlations were significant in plasma ($r = 0.503\text{--}0.984$), whereas all ten were significant in mucus ($r = 0.616\text{--}0.988$). The presence of significant associations among all biomarkers in mucus indicates that antioxidant capacity and protein oxidation indicators varied in a more coordinated manner in this matrix. This pattern could be related to its direct exposure to the external environment and the local interaction between oxidant production and antioxidant defences at the body surface. In plasma, by contrast, biomarkers may be influenced by processes originating in different organs and tissues, which could explain less generalised covariation. Nevertheless, these correlations do not demonstrate that all biomarkers share the same regulatory mechanism.

The absence of correlations between the two matrices suggests that mucus does not directly reflect the systemic redox status observed in plasma. Body weight was not correlated with any of the biomarkers evaluated.

Conclusion

Skin mucus showed greater antioxidant capacity and a higher AOPP concentration than plasma, suggesting more intense local redox activity. The absence of correlations between the two matrices, together with the greater covariation of biomarkers in mucus, indicates that plasma and mucus represent distinct physiological compartments. Therefore, mucus should not be considered a direct substitute for plasma, although it may constitute a complementary and less invasive matrix for assessing local antioxidant and oxidative processes in wild adult Atlantic bluefin tuna.

FEEDS AT THE FRONTIER OF AQUACULTURE: HOW TO DEAL WITH EMERGING SPECIES FROM A FEED PRODUCERS' PERSPECTIVE

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Introduction

Despite the high diversity of marine food fish, aquaculture is limited to relatively few species. Many marine species remain dependent on wild capture. Securing long-term supply therefore requires the successful establishment of new aquaculture species. Atlantic cod (*Gadus morhua*) and Atlantic halibut (*Hippoglossus hippoglossus*) are promising species for large-scale production. In contrast to Atlantic salmon (*Salmo salar*), cod and halibut still face biology- and nutrition-driven production challenges that limit predictable growth and quality, requiring adaptive feed development and close producer-farmer collaboration.

Challenges, materials, and methods

In cod, nutritional imbalances are associated with skeletal deformities, liver alterations, and intestinal abnormalities, particularly during early development and under intensive rearing conditions (Pedro et al., 2025). In halibut, impaired eye migration, ambicoloration, and skin wounds are major bottlenecks, with diet composition strongly influencing development outcomes (Sæle et al., 2003; Hamre et al., 2007).

To address these challenges, feed development was conducted through a stepwise, trial-based collaboration between Aller Aqua and the farmers Havland and Sterling Halibut. Diets were refined to reflect species-specific nutritional sensitivities, improving performance, health, and sustainability.

Cod feed development followed a consecutive research approach. A baseline trial in 2022 at Aller Aqua Research evaluated nutrient requirements and pellet quality under controlled conditions. This was followed by a trial series in 2025 under recirculating aquaculture system (RAS) conditions, assessing dietary effects on water quality, growth, and skeletal deformities, with feed formulations adjusted based on biological responses. In 2026, a flow-through trial evaluates feed performance across production systems and the inclusion of alternative raw materials, with growth, deformities, and gut health monitored through regular sampling.

Halibut feed development applied a similar stepwise approach, focusing on protein source suitability. Following earlier indications of limited tolerance for plant ingredients, a land-based by-product protein was tested in 2023. A second alternative protein was added in 2024 to increase fishmeal replacement, and higher inclusion levels of the by-product were evaluated in 2025. Growth performance, health indicators, and sustainability metrics were assessed at regular sampling intervals.

Results

Cod feed development under RAS conditions established a functional baseline feed for land-based production, supporting stable performance and firmer feces that contributed to improved water quality. Skeletal deformities, including neck cracks, were observed but were assessed to be within the normal range for cod during grow-out phase. There is no clear evidence that deformities occur more frequently under RAS compared with other production technologies. The nutritional and physiological causes of neck cracks remain unclear and are therefore addressed as a general challenge for cod farming. An ongoing flow-through trial provides complementary data on feed performance and health parameters, across land-based systems and on the use of alternative raw materials.

Halibut feeding trials demonstrated strong potential for land-based protein sources into grower diets. Inclusion of a land-based by-product in 2023 resulted in similar growth performance between both diets. A combination of the by-product and another land-based protein source in 2024 resulted in good growth performance and 33% less wound prevalence in the test diet. Increasing the inclusion of the by-product from 2024 to 2025 resulted in 15.57% lower FCR in the test diet compared to control diet. The test diet consistently reduced the prevalence of wounds, with 40.58% less wound prevalence in the test

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diet compared to the control diet in 2025. The latest results indicate an association between eye migration, ambicoloration, and growth performance. The proportion of fully ambicolorated fish was 40% higher among individuals with incorrect eye migration compared to fully ambicolorated fish with correct eye migration. In addition, average body weight was 11.45% lower in fish with incorrect eye migration compared to fish with correct eye migration. Improved feed sustainability was reflected by a 27.52% reduction in FIFO through inclusion of the tested novel ingredients from 2025, with a further 26.09% reduction associated with increased inclusion rates of novel protein sources compared to 2024.

Outlook

Scaling cod and halibut production to 10,000 tons requires species-specific feeds that support growth, skeletal integrity, and overall health. Norway's goal of 25% self-sufficiency in sustainable aquafeed ingredients by 2034 further highlights the need for alternative raw materials. This work demonstrates how targeted feed innovation can enable emerging marine species and be transferable to species with similar nutritional requirements, such as spotted wolffish (*Anarhichas minor*).

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METABOLIC ROLES OF VITAMINS AND MINERALS IN FISH GUT MICROBIOTA: A MOLECULAR NUTRITION PERSPECTIVE

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Micronutrients, like vitamins and trace minerals, help to regulate fish physiology, immunity, and growth by engaging in direct metabolic processes and regulating interactions with the gut microbiota. Recent study has shown that these micronutrients influence host-microbiota metabolic networks via transcriptional regulators such as FXR, as well as epigenetic and antioxidant pathways. Environmental stresses, such as ammonia and chemical pollutants, can disrupt these networks, lowering nutrient consumption and threatening host resilience. High-resolution characterization of microbial diversity, gene expression, and metabolic responses to dietary interventions has been made possible by omics technologies: metagenomics, transcriptomic, and metabolomics. These technologies provide mechanistic insights into dose-dependent micronutrient effects. Precision nutrition techniques such as the best vitamin and mineral supplements, nanostructured nutrients, and environmental control can enhance growth performance, immune function, and metabolic health in aquaculture species. In order to support the creation of integrative evidence-based feeding strategies in aquaculture, this review summarizes molecular, microbial, and physiological evidence, points out important knowledge gaps, and suggests future research directions.

IN OVO DELIVERY OF IMMUNOSTIMULANTS AS AN APPROACH TO REDUCE RAINBOW TROUT LARVAE MORTALITY

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Introduction

The high and unpredictable mortality of fish larvae in the first weeks after hatching remains a challenging problem for the aquaculture sector. Both scientific evidence and hatchery experience across a range of fish species, support the hypothesis that detrimental fish–microbe interactions are contributing to this problem. Among the recommended strategies to prevent these health problems is the induction of larvae immune capacity. We are investigating an innovative approach to evaluate the potential of stimulating the developing embryo immune system at the earliest possible point by delivering immunostimulants directly to fish eggs. This novel method employs a molecular transporter that can deliver the immunostimulant across the chorion and vitelline membrane in a bath immersion-style treatment, circumventing the need to microinject individual eggs/embryos. The approach is potentially scalable, enabling the simultaneous treatment of large number of eggs in a versatile and cost-effective manner suitable for its application in the industry.

Materials and Methods

As a starting point, rainbow trout (*Oncorhynchus mykiss*) eggs were exposed to a well-established immunostimulant, widely used in fish, with proven potential to trigger both humoral and cell-mediated immune responses and to provide protection against bacterial, viral, and parasitic infections. Different concentrations of the immunostimulant and corresponding controls were tested in unfertilized eggs to assess potential toxicity. Several incubation time points were evaluated. Previous trials established conditions able to preserve eggs viability during the immersion treatments [Xu et al, 2023].

Results and Discussion

Results revealed that the fluorescently labelled immunostimulant could be incorporated into trout unfertilized trout eggs. However, the observed fluorescence was weak, and incubation periods exceeding prolonged incubation 48 h were required, potentially compromising egg viability. To overcome this limitation, a new molecule was developed by coupling the immunostimulant to a molecular transporter at its 5' end using click chemistry and attaching a fluorescent label at its 3' end. This specific design enabled tracking of compound uptake by the egg, which was confirmed by fluorescence microscopy after 42 h of incubation, with fluorescence detected in the ooplasm. Sperm collected from rainbow trout neomales was used to fertilize the eggs. Embryos were reared in static, oxygenated water at 10°C under a simulated natural photoperiod in a controlled-temperature room. Eyed rates were similar between control and treated groups, indicating no apparent detrimental effect of the treatment on early embryos. Development was followed until 17 days post-hatching (dph). At 10 dph, larvae were challenged with *Yersinia ruckeri*, a bacterium that causes enteric red mouth disease. To induce a systemic infection, the bacterium was administered by injection into the *Duct of Cuvier*, with each alevin receiving 6.9×10^3 colony-forming units (CFU). Survival rates were close to 6% in infected fish, irrespective of the previous immersion treatment, compared with 70% in saline-injected controls. These results indicate that a lower bacterial dose should be used in future challenge experiments. Overall, this study provides preliminary evidence for a promising *in ovo* bath immersion approach for delivering immunostimulants to fish eggs and potentially stimulating the developing embryonic immune system. Future work will focus on refining immunostimulant dosage and incubation duration and elucidating the pathways triggered by the treatment. Ultimately, we plan to expand this technology to other aquaculture species.

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INNOVATIVE ANTIBODIES FOR HEALTH BIOMARKERS DETECTION IN AQUACULTURE

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Introduction

Aquaculture often suffers from rapidly spreading epizootics with high mortality, requiring strict biosecurity, early detection, and rapid response. However, the lack of sensitive and rapid diagnostic tools limits effective disease management. Antibodies are excellent biorecognition molecules that can recognize their target with remarkable specificity and high affinity, making them ideal components for biosensors. Unlike conventional mammalian antibodies, sharks produce a unique type of antibody called immunoglobulin new antigen receptor (IgNAR), composed only of heavy chains. Its variable domain, known as VNAR, is the smallest naturally occurring antigen-binding domain in the animal kingdom. Despite their small size, VNARs combine high specificity with exceptional stability and can recognize cryptic epitopes that are often inaccessible to conventional antibodies, making them highly attractive for diagnostic and therapeutic applications (Cheong et al., 2020; Zielonka et al., 2015).

Traditionally, antibody production relies on animal immunization. However, ethical concerns and regulations such as EU Directive 2010/63/EU encourage the use of animal-free alternatives whenever possible. One of the most powerful of these alternatives is phage display technology. Using this method, millions of different antibody variants can be displayed on the surface of bacteriophages, allowing researchers to rapidly identify those that bind to a target of interest.

This work focuses on building a naïve shark VNAR antibody library using phage display methodology, enabling the subsequent generation of recombinant antibodies against fish health biomarkers for real-time, on-farm monitoring via the development of biosensors.

Methodology

Buffy coats from blood of five adult naïve nurse sharks were used for total RNA extraction. Purified RNA was reverse-transcribed to cDNA using VNAR framework region (FR) 4-specific primers. The VNAR repertoire was PCR-amplified with primers targeting FR1 and FR4 conserved regions, then cloned into the phagemid vector pADL-23c, in-frame with the phage coat protein g3p between two SfiI restriction sites. The resulting purified constructs were used to transform *E. coli* TG1 electrocompetent cells by electroporation. Thirty-two independent electrotransformations were performed, pooled, and plated onto 16 large square bioassay dishes containing 2×YT agar medium supplemented with 2% glucose and 50 µg/mL carbenicillin. Transformants were grown at 30 °C for 12 h. Following incubation, colonies were gently scraped, pooled, and stored at -80 °C as primary glycerol stocks of the library (TG1 library). Aliquots of TG1 cells harbouring the recombinant phagemids were infected with VCSM13 helper phage, at a multiplicity of infection (MOI) of 20:1, to generate the final library stock of infectious phages (phage library) displaying the VNAR antibodies repertoire.

Results and discussion

The VNAR TG1 library size was determined by counting colony-forming units (CFU) on selective agar plates after transformation. The resulting library yielded around 3×10^7 transformants. Viability assays of frozen aliquots of the TG1 library demonstrated a high post-thaw recovery of approximately 5×10^{10} CFU/mL (ca. 83% viability), ensuring the preservation of the library's original clonal diversity. Additionally, PCR analysis confirmed that the transformed colonies carried recombinant VNAR phagemids. To analyze the clonal diversity of the library prior to selection of VNAR antibodies, deep sequencing of the whole library is being performed using next-generation sequencing (NGS). This approach aims to assess the number of viable and functional VNAR variants, detect potential biases, estimate redundancy, and characterize the CDR3 distribution.

Preliminary results demonstrate the generation of a viable VNAR library suitable for screening against biomarkers of interest. Following successful TG1 transformation, stock recovery, and helper phage rescued-phage production, future steps will involve library screening through targeted antigen incubation. Isolated candidates will subsequently be produced at large scale and validated for their potential application in biosensors.

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LOW-VALUE BIOMASS-DERIVED FUNCTIONAL ADDITIVES IMPROVE GROWTH AND SURVIVAL OF *Sparus aurata* POST-LARVAE DURING A NATURAL *Photobacterium damsela* subspecies *piscicida* OUTBREAK.

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Introduction

The increasing use of alternative ingredients and higher stocking densities in aquaculture are contributing to elevated disease pressure, particularly during early developmental stages. Gilthead seabream (*Sparus aurata*) post-larvae are especially susceptible to bacterial infections such as *Photobacterium damsela* subsp. *piscicida* (*phdp*), coinciding with maturation of the immune system. In this context, the valorisation of low-value biomass and agro-industrial by-products as functional feed additives represents a promising strategy, contributing to both sustainability and improved growth, health, and disease resistance in aquaculture (Zuberi *et al.*, 2024).

Materials and Methods

Three experimental diets were tested: a control diet (CTRL), a diet supplemented with a pomegranate by-product (PG0.05; 500 ppm), and a diet including a marine algae-derived supplement (Marine; 2.65%). Gilthead seabream post-larvae were reared from 45 to 115 days post-hatch (dph) in triplicate tanks (600 larvae per 200 L). During the trial, a natural outbreak of *phdp* occurred and was confirmed by qPCR from symptomatic fish and bacterial isolates. All groups were exposed under identical rearing conditions. Growth performance (final weight and length) and survival were assessed after the outbreak, considering a 5-day mortality-free period to confirm the end of the infection event. Survival prior to the outbreak was also calculated. Samples of liver and spleen were collected for gene expression analysis, and whole larvae were preserved for immunohistochemistry.

Results and Discussion

No significant differences in survival were observed among groups prior to the outbreak, indicating that dietary treatments did not affect normal post-larval development. However, following the infection event, fish fed supplemented diets showed significantly ($p < 0.05$) improved growth performance, both in final weight and length (Fig. 1A, B), compared to the control group. In addition, survival analysis revealed higher survival rates in supplemented groups (PG0.05 and Marine), compared to CTRL (Fig. 1C), with significant differences detected by Kaplan–Meier analysis (log-rank test, $p < 0.05$). The improved survival observed during the outbreak suggests that these additives enhance resilience under disease conditions, likely through the action of natural bioactives known to stimulate immune effectors (e.g., antibodies, cytokines and antimicrobial peptides), activate innate immune cells, and reduce tissue damage via antioxidant activity (Rahul Sandeep *et al.*, 2025; Abdul Kari, 2025).

Although the lack of controlled infection conditions limits causal inference, all groups were exposed under identical rearing conditions, supporting the consistency of the observed differences. Overall, these findings highlight the potential of low-value biomass-derived additives to enhance larval robustness under commercial aquaculture conditions. Ongoing analyses (gene expression and immunohistochemistry) will further elucidate the mechanisms underlying these effects.

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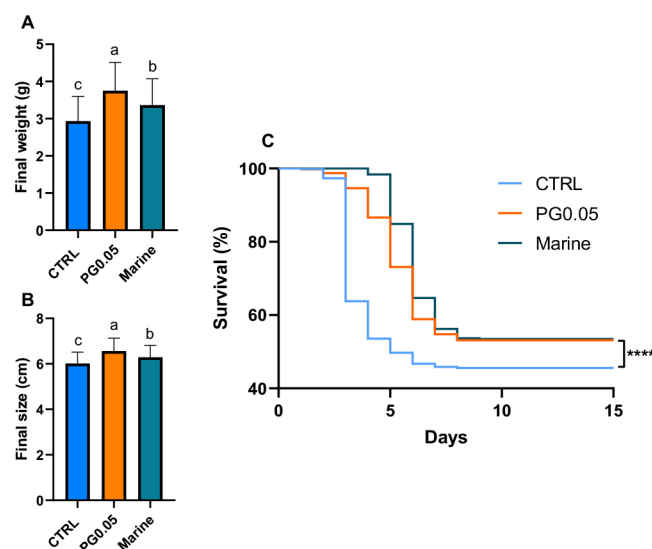


Figure 1. Growth performance and survival of gilthead seabream (*Sparus aurata*) post-larvae fed diets supplemented with low-value biomass-derived additives during a natural *Photobacterium damsela* subsp. *piscicida* outbreak. (A) Final body weight and (B) total length at 115 dph. (C) Kaplan–Meier survival curves. CTRL: control diet; PG0.05: pomegranate by-product (500 ppm); Marine: algae-derived supplement (2.65%). Data are presented as mean $\pm$ SD ($n = 3$ tanks per treatment). Different letters indicate significant differences (ANOVA, Tukey’s test, $p < 0.05$). Survival differences were analysed using the log-rank (Mantel–Cox) test.

AEROBIC EXERCISE AND LOW FISHMEAL DIET WITH TAURINE ADDITION MAY ENHANCE MUSCLE HYPERPLASIA WHILE MAINTAINING GROWTH PERFORMANCE AND ROBUSTNESS IN EUROPEAN SEA BASS FINGERLING

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Introduction

Improving fish growth in aquaculture remains a major challenge, which must now be addressed together with the need for more sustainable production strategies. In this context, treatments during fingerling period have been explored to obtain European sea bass (*Dicentrarchus labrax*) juveniles with better growth and quality. On one hand, the reduction at 12.5% of fish-derived ingredients in E. sea bass juveniles improved growth and feed utilization when taurine (Tau) supplementation was applied (Martins et al., 2018). On the other hand, moderate and continuous exercise in E. sea bass fingerlings have been determined to produce greater aerobic and protein synthesis capacities, and higher protein retention efficiency (Perelló-Amorós et al., 2024). Then, the main objective of the present work is to combine exercise and Tau supplemented low fish meal diet to produce fingerlings with better robustness. A growth trial with different diets and swimming condition was performed. After two month fed with a commercial diet at the end of the trial, a swim endurance test was carried out.

Material and Methods

European sea bass fingerlings (5.49 ± 0.03 g) were pit-tagged and distributed among three replicate tanks per condition (2 g/L) and subjected to voluntary (VS) or sustained swimming (SS; 1.5 BL/s). For eight weeks, fish were fed one of three diets: high fishmeal (HFM; 25%), low fishmeal (LFM; 10%), or LFM supplemented with 0.9% taurine (LFM+TAU). Growth was assessed at weeks 0, 4, and 8. At the end of the trial, biometric indexes were determined, and tissues were collected for gene expression analyses and muscle histological analyses.

After feeding trial, swim endurance tests were conducted in ten fish from each of the HFM-SS, LFM+TAU-VS, and LFM+TAU-SS groups using an intermittently closed respirometer (Loligo® system). Fish were tested individually, one per day, and habituated for at least 30 min at $0.25 \text{ BL} \cdot \text{s}^{-1}$ until oxygen consumption (MO_2) stabilized. Maximum metabolic rate (MMR) was determined from maximum O_2 consumption, while routine metabolic rate (RMR) was calculated from the exponential curve at zero speed. Aerobic metabolic scope (AMS) was reckoned as the difference between MMR and RMR, and critical swimming speed (U_{crit}) was obtained from the speed at exhaustion.

Statistical analyses (SPSS v30) included normality testing (Shapiro-Wilk), homoscedasticity (Levene's test), one- or two-way ANOVA with Tukey's 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.

		HFM-VS	HFM-SS	LFM-VS	LFM-SS	LFM+TAU-VS	LFM+TAU-SS	P-value
Biometric parameters	Final Weight (g)	20.59 ± 0.24	21.3 ± 0.26	20 ± 0.25	20.75 ± 0.27	20.37 ± 0.25	21.18 ± 0.22	A: 0.032 D: 0.459 A x D: 0.987
	4-8 weeks SGR	1.80 ± 0.05 ^b	1.93 ± 0.06 ^b	1.54 ± 0.08 ^a	1.69 ± 0.01 ^a	1.63 ± 0.11 ^{ab}	1.71 ± 0.11 ^{ab}	A: 0.120 D: 0.027 A x D: 0.929
	0-8 weeks SGR	2.04 ± 0.03	2.13 ± 0.01	1.99 ± 0.04	2.01 ± 0.04	1.98 ± 0.07	2.08 ± 0.07	A: 0.008 D: 0.166 A x D: 0.127
Somatic indexes	MFI	5.49 ± 0.18 ^b	5.47 ± 0.23 ^b	5.28 ± 0.24 ^{ab}	5.22 ± 0.24 ^{ab}	4.94 ± 0.31 ^a	4.73 ± 0.29 ^a	A: 0.629 D: 0.028 A x D: 0.912
	MSI	36.38 ± 0.7 ^{ab}	36.91 ± 0.86 ^{ab}	35.39 ± 0.62 ^a	35.22 ± 0.48 ^a	36.89 ± 0.72 ^b	38.88 ± 0.57 ^b	A: 0.162 D: 0.002 A x D: 0.291

Table 1: Somatic growth parameters of fish fed with high fishmeal (HFM), low fishmeal (LFM) or supplemented with Taurine (LFM+TAU) and under voluntary swimming (VS) or sustained swimming (SS) for 8 weeks.

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Results and Discussion

At the end of growth trial the final body weight was highest in HFM-SS and LFM+TAU-SS (Table 1), both greater than other groups. During the final month of the trial (weeks 4–8), specific growth rate (SGR) was significantly higher in fish fed the HFM diet, followed by those fed the LFM+TAU diet. However, over the entire two-month trial (weeks 0–8), the swimming groups exhibited significantly higher SGR values. Fish fed the LFM+TAU diet had a significantly higher muscle somatic index and lower mesenteric fat content. The histological analysis showed that the fish fed LFM+TAU had a significantly both lower fiber density and fiber cross-sectional area than HFM and LFM groups, suggesting enhanced hyperplasia process induced by taurine. In contrast to that observed in gilthead sea bream (Moya et al., 2019), exercise did not modify the fibres morphology. Sample size will be increased in future analyses to confirm these findings.

No differences in swimming performance (Ucrit) or oxygen consumption were observed among fish from the three selected dietary and exercise conditions. Reducing dietary fishmeal inclusion and supplementing the diet with taurine did not compromise fish robustness. Aerobic capacity improvement was already noticed in E. sea bass juveniles under exercise condition (Perelló et al., 2024). However, this upgrade was not observed at the fingerling stage. The absence of differences may partly reflect the resilience of fish following their transfer to commercial rearing conditions, which could have allowed the LFM+TAU group to maintain performance comparable to that of the HFM group. Overall, sustained swimming enhanced growth, while the LFM+TAU diet achieved performance comparable to the HFM diet under swimming conditions. Taurine also increased the muscle somatic index and reduced mesenteric fat, supporting its potential to reduce fishmeal inclusion in European sea bass diets.

Acknowledgment

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ENVIRONMENTAL CHARACTERISATION OF SARDINIAN COASTAL LAGOONS TO SUPPORT SUSTAINABLE AQUACULTURE PLANNING

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Introduction

Coastal lagoons are dynamic transitional environments that support high biological productivity and a wide range of ecological functions, while also supporting important ecosystem services such as fisheries and aquaculture. At the same time, their environmental variability and sensitivity to climatic and anthropogenic pressures make sustainable management particularly challenging (Pérez-Ruzafa et al., 2019). Managing traditional aquaculture and capture fisheries is considered the main instruments for preserving lagoons ecological features and preventing habitat degradation, in both environmental and socioeconomic terms (Cataudella et al., 2015). Sardinia, the second-largest island in the Mediterranean Sea, hosts numerous coastal lagoons distributed along its coastline. These systems are closely linked to artisanal fisheries and aquaculture and combine high ecological value with important productive functions. Several of them are included in the regional planning framework for Allocated Zones for Aquaculture (AZAs) (Macias et al., 2015) covering both marine and inland waters. FAO guidelines emphasise that spatial designation should be complemented by environmental characterisation, reference conditions, and monitoring capable of evaluating environmental performance over time (FAO, 2025). Within the Sardinian regional project AZA II, funded under the 2021–2027 Regional European Maritime, Fisheries and Aquaculture Fund, an environmental characterisation programme is being implemented to support the sustainable management of lagoon aquaculture. Five coastal lagoons (Cirdu, Cabras, Corru Mannu, Tortoli, and San Giovanni Muravera) are being seasonally monitored during 2025–2026, representing the first monitoring effort in the area to combine such extensive temporal and spatial coverage with targeted integrated environmental assessments. The objective is to establish a robust environmental baseline and provide evidence required to evaluate environmental status, identify potential risks, and support the assessment of sustainable productive carrying capacity.

Materials and Methods

Seasonal monitoring was conducted during 2025–2026 in five Sardinian coastal lagoons. Spatially distributed grid stations were selected to provide broad coverage of each lagoon and capture major environmental gradients (46 stations in total), while targeted stations (24 stations in total) were established for integrated water, sediment and biological sampling in areas representative of different environmental settings, ecological and aquaculture conditions. At these targeted stations, sampling encompassed the water column, sediment, benthic communities, and environmental DNA (eDNA). Stations were selected in proximity to aquaculture facilities, fish barriers (lavorieri), lagoon inlets, regional monitoring sites, and areas identified through historical information. Water variables included temperature, salinity, dissolved oxygen, pH, turbidity, chlorophyll-a, particulate matter and nutrients. Sediment characterisation included redox potential, grain size, organic matter, chlorophyll-a and elemental composition, together with the identification of macrozoobenthos and meiofauna and the characterisation of microbial communities through eDNA. The resulting environmental and biological descriptors are being jointly evaluated to characterise lagoon-specific ecological conditions, identify potential risk patterns, and provide the empirical basis for subsequent carrying capacity assessment.

Results

The ongoing monitoring confirmed spatial and seasonal variability within and among the five lagoon systems. Water-column parameters differed among lagoons and between seasons, particularly for temperature, salinity, dissolved oxygen, turbidity and chlorophyll-a, reflecting differences in marine exchange, freshwater influence and confinement. Sediment measurements also showed marked spatial heterogeneity, with redox conditions indicating that benthic compartments may respond differently to environmental changes than the overlying water column. Concerning the biological component, 198 macrozoobenthic samples have been analysed and approximately 200 taxa identified, including around 160 identified at species level. The combination of broad spatial coverage and targeted integrated stations is therefore providing sufficient resolution to distinguish intrinsic lagoon variability from potentially critical environmental conditions.

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Discussion

The results provide an environmental baseline for moving lagoon monitoring beyond descriptive characterisation towards operational assessment of environmental performance and aquaculture carrying capacity. A spatially explicit baseline can support early detection of extreme events, which are particularly relevant in shallow, semi-confined transitional systems. Restricted water exchanges and pronounced environmental variability can amplify disturbances such as heat waves, hypoxia or anoxia, and eutrophication episodes. Comparing repeated observations with baseline conditions can help identify such changes before they result in production losses or ecosystem degradation. Repeated observations can also help define the environmental state of each lagoon, evaluate trends, and provide an empirical basis for estimating sustainable productive carrying capacity.

For competent authorities and concessionaires, the monitoring framework can therefore act as a decision-support tool linking AZA planning, environmental quality, and aquaculture management. The integration of physical, chemical, sedimentary and biological information also provides a basis for developing site-specific monitoring and management thresholds and abiotic/biotic indices. A critical next step is to translate early warnings into a clear operational response chain, including quality-control checks, rapid confirmation or resampling, alert levels, assigned responsibilities, communication pathways and appropriate management actions. Such a framework would strengthen the connection between environmental monitoring and aquaculture management and improve the capacity to respond to increasing climatic and anthropogenic pressures.

Acknowledgment

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FROM SPATIAL SUITABILITY TO ADAPTIVE MANAGEMENT: A MULTI-ENVIRONMENT APPROACH TO ALLOCATED ZONES FOR AQUACULTURE IN SARDINIA (ITALY)

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Introduction

The sustainable development of aquaculture requires spatial planning approaches capable of reconciling production objectives with environmental protection and competing uses of aquatic space. Allocated Zones for Aquaculture (AZA) have emerged as an important planning instrument to identify areas where aquaculture can be developed while reducing spatial conflicts and supporting an ecosystem-based approach. Spatial multi-criteria approaches have demonstrated the potential to integrate environmental suitability and anthropogenic constraints in support of aquaculture spatial planning (Brigolin et al., 2017; Porporato et al., 2020). However, AZA planning has traditionally focused predominantly on marine and coastal environments, while integrated approaches encompassing different aquatic environments and production systems remain comparatively limited. This study presents the methodology developed for the identification of Allocated Zones for Aquaculture in Sardinia, Italy, as part of the regional AZA planning process. The objective was to develop a spatially explicit and reproducible framework integrating environmental suitability, spatial constraints and competing uses, while accounting for the specific requirements of different aquaculture systems and potential farming species. A further objective was to establish the basis for a planning framework capable of evolving from spatial allocation towards long-term environmental monitoring and adaptive management.

Methodology

The methodology was developed within Multi-Criteria Analysis (MCA) framework. Environmental, oceanographic, ecological, and anthropogenic datasets were integrated with regulatory and spatial constraints. Relevant criteria were transformed into spatial layers and combined through a structured suitability assessment, building on previous applications of spatial multi-criteria evaluation to aquaculture site selection and zoning (Brigolin et al., 2017; Porporato et al., 2020).

A key feature of the approach was the differentiation of the analysis according to the characteristics of the main aquatic environments and production systems. Marine waters, coastal lagoons, and freshwater lakes were analysed using environment-specific criteria. In addition, species-specific suitability was assessed for different potential farming species within each environment, allowing areas to be evaluated not only according to their general compatibility with aquaculture, but also according to their potential suitability for alternative production options. Spatial constraints were subsequently integrated to identify areas where aquaculture development was excluded or subject to specific limitations. Protected areas, sensitive habitats, navigation, fisheries, tourism, coastal infrastructures, and other maritime and inland uses were considered within the planning framework. The resulting spatial analysis was then incorporated into the regional planning process and subjected to institutional and stakeholder review.

Results and discussion

The application of the methodology resulted in the identification of 94 Allocated Zones for Aquaculture across Sardinia, comprising 63 marine zones, 26 lagoon zones, and 5 freshwater lake zones. The resulting framework extends the AZA concept beyond its traditional predominantly marine application and provides a common spatial planning structure for marine, transitional, and freshwater aquaculture. The species-specific suitability assessment adds a further dimension to the planning process by linking place, environmental conditions, and potential production systems. This allows the spatial planning framework to support aquaculture diversification rather than identifying areas solely according to generic suitability for aquaculture. This approach builds on previous work demonstrating the value of combining environmental suitability and production-related information in aquaculture spatial planning (Porporato et al., 2020), while extending the concept across multiple aquatic environments and production systems.

A further distinctive element of the Sardinian approach is its integration into a formal regional planning process. The resulting spatial framework constitutes the basis of a regional AZA Plan intended to guide future aquaculture development while supporting environmental safeguards and the coexistence of aquaculture with other uses. The Plan is currently undergoing Strategic Environmental Assessment (SEA) and Appropriate Assessment (AA) procedures, providing an additional step for evaluating potential environmental effects and refining the proposed zoning framework.

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The planning process is also being continued through a complementary project funded under the Regional European Maritime, Fisheries and Aquaculture Fund. This follow-up phase is designed to move from spatial allocation towards adaptive management of the identified zones, including the development of Environmental Monitoring Programmes for marine and lagoon areas, the assessment of carrying capacity, the identification of suitable Blue Economy development models, and the definition and monitoring of indicators associated with implementation of the Plan. This progression represents a shift from static spatial zoning towards a dynamic planning cycle, in which environmental performance, production potential and interactions with other activities can be monitored and used to inform future updates of the AZA Plan. The Sardinian case demonstrates how the integration of multi-environment and species-specific suitability assessment with environmental assessment, monitoring, carrying capacity and adaptive management can provide a transferable framework for supporting sustainable and diversified aquaculture development.

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SHIELD: STRENGTHENING MANILA CLAM'S RESILIENCE TO CLIMATE CHANGE-RELATED STRESS AND PATHOGENS

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Introduction

Manila clam (*Ruditapes philippinarum*) aquaculture is increasingly threatened by the combined effects of *Perkinsus* infections and climate-driven heatwaves. Current strategies largely focus on selective breeding for resistant stocks, but this approach may overlook other biological mechanisms influencing resilience. Two processes are particularly relevant: transgenerational effects of pathogen exposure and early-life microbial interactions. SHIELD is a Marie Skłodowska-Curie Actions Postdoctoral Fellowship project, hosted at the University of Padova in collaboration with CIIMAR (Porto), that investigates both mechanisms to identify new, practical strategies to improve clam performance under environmental stress.

WP1: Transgenerational Effects of *Perkinsus* Infection

While the immediate effects of *Perkinsus olsenii* on clam physiology and reproduction are documented, whether parental infection leaves a heritable imprint on offspring resilience has never been systematically studied in Manila clams. WP1 addresses this by experimentally infecting adult clams before gonadal maturation and crossbreeding them to produce four offspring groups with different parental infection histories. Larvae and juveniles from each group are reared and subsequently tagged and seeded in the Venice Lagoon, where *Perkinsus* is endemic. Growth rates, survival, infection intensity (RFTM and qPCR), microbiota composition (16S rRNA), gene expression (RNA-seq), and chromatin accessibility (ATAC-seq) are assessed at multiple time points to capture both the immediate and longer-term consequences of parental exposure. A specific focus is placed on comparing maternal versus paternal transmission of any observed effects.

WP2: Microbiota Priming During Early Larval Development

Hatcheries routinely raise bivalve larvae in UV-treated, near-sterile seawater, a practice that limits pathogen exposure but may also deprive larvae of the microbial stimuli needed for normal immune development. WP2 tests whether exposing larvae to microbiota-enriched (ME) seawater, conditioned by healthy donor clams from a natural environment, during the first 10 days of hatchery rearing leads to better performance later in life. Control larvae are maintained in standard UV-treated conditions. Once juveniles reach approximately 4 months of age, will be seeded at a farming site in the Venice Lagoon with known *Perkinsus* and *Vibrio* pressure. Preliminary data from the larval phase show that ME-exposed larvae grew faster (23.25 $\mu\text{m day}^{-1}$ vs. 14.21 $\mu\text{m day}^{-1}$ in controls) and produced approximately 2.5 times more larvae at the nursery transition, pointing to an early and measurable benefit of microbiota contact even before any immune challenge.

Expected Outcomes

By integrating genomic, epigenomic, and microbiome approaches with field and laboratory experiments, SHIELD aims to identify the biological mechanisms behind clam resilience and translate them into actionable recommendations for hatchery management. If parental *Perkinsus* exposure is shown to affect offspring fitness, this would open new avenues for selective broodstock management. If ME seawater shows to strongly improve disease resistance and thermal tolerance in juveniles, it would offer hatcheries a simple protocol adjustment with direct relevance for clam production in a changing climate. Results from both WPs will be made available through open-access publications and shared directly with industry partners.

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EARLY-LIFE MICROBIOTA EXPOSURE ENHANCES PERFORMANCE AND RESILIENCE IN MANILA CLAM

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Introduction

Early microbial exposure is increasingly recognized as an important factor shaping host development and immune function in marine organisms. In bivalve hatcheries, however, larvae are typically reared in UV-treated seawater, resulting in reduced microbial diversity during early life stages. Whether this matters in the long run is still an open question for *Ruditapes philippinarum*, a species whose aquaculture is already under severe pressure from *Perkinsus* infections and climate-driven heatwaves. Here we present the preliminary results from WP2 of SHIELD, a Marie Skłodowska-Curie Actions Postdoctoral Fellowship project, testing whether exposing hatchery larvae to microbiota-enriched (ME) seawater, conditioned by healthy donor clams, produces juveniles with better growth performance and, ultimately, greater resilience to environmental stressors.

Materials and Methods

Broodstock from a *Perkinsus*-free population were induced to spawn at Naturesdulis (Goro, Italy). Resulting larvae were divided into two groups from day 1: an ME group, exposed for 10 days to seawater conditioned by donor clams from a natural, pathogen-free environment placed upstream of the larval tanks, and a control group maintained in standard UV-treated seawater. Shell length was measured at day 8 and day 13 post-fertilisation. Larval volume was recorded at the transition from conical tanks to downwelling baskets, after which larvae were distributed into three replicate baskets per treatment for the nursery phase. Once juveniles reach approximately 4 months of age, they are transferred to a *Perkinsus*-endemic site (Chioggia, Venice Lagoon Italy) for a natural field challenge throughout the summer. A separate cohort kept in hatchery conditions is later exposed to a simulated heatwave under controlled laboratory conditions. Microbiota composition (16S rRNA gene sequencing), gene expression (RNA-seq), and epigenetic modifications (ATAC-seq) will be characterised at key time points across both challenges.

Results

At day 8, ME larvae were slightly smaller on average than controls (184.2 $\pm$ 14.0 μ m vs. 190.1 $\pm$ 36.9 μ m) but showed considerably more uniform size distribution (CV 7.6% vs. 19.4%). By day 13, ME larvae had overtaken controls in mean shell length (231.0 $\pm$ 10.5 μ m vs. 218.4 $\pm$ 14.2 μ m), corresponding to growth rates of 23.25 μ m day⁻¹ and 14.21 μ m day⁻¹ respectively. At the time of transfer to downwelling baskets, larval volume was approximately 14 ml in the ME group compared to 5.5 ml in controls, roughly 2.5 times more larvae available for the nursery stage. Juveniles from both groups are currently growing in replicate tanks at Naturesdulis (Goro, Italy); heatwave and field exposure experiments are planned for the coming months.

Discussion

Even at this early stage, the WP2 data suggest a noticeable benefit of ME exposure: faster and more uniform larval growth, and substantially higher larval yield at the nursery transition. For hatchery operators, a 2.5-fold increase in larval volume at transfer represents a meaningful gain.

These results indicate that early microbial exposure can enhance larval growth performance, improve batch uniformity, and substantially increase production yield at the hatchery stage. Importantly, these benefits are achieved without changes to infrastructure, making this approach directly applicable in aquaculture settings. Ongoing experiments will determine whether these early advantages persist under heat stress and pathogen exposure.

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ENHANCING PERFORMANCE OF LOW FISHMEAL DIETS IN RAINBOW TROUT *Oncorhynchus mykiss* UNDER HIGH DENSITY CONDITIONS THROUGH UMAMI-MEDIATED GUT SENSING MECHANISMS

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Introduction

The reduction of fishmeal (FM) inclusion in aquafeeds is a key priority due to its limited availability, cost volatility, and sustainability concerns. However, lowering FM levels often impairs feed intake and performance in carnivorous fish. Fish possess highly sensitive gustatory systems capable of detecting amino acids (AA), key drivers of umami taste associated with protein-rich ingredients. Importantly, taste receptors are also expressed along the gastrointestinal tract, where they participate in nutrient sensing and regulation of digestion and appetite via gut–brain signalling pathways (Angotzi et al., 2022). Therefore, dietary strategies targeting both oral and post-ingestive chemosensory mechanisms may help maintain performance in low FM diets.

Materials and methods

A 91-day feeding trial was conducted with rainbow trout (initial body weight: 29.1 ± 2.1 g) under high-density conditions (29 to 53 kg m⁻³ at the start and end of the trial, respectively) in aerated freshwater (14.2°C). Fish were distributed into triplicate tanks (50 fish tank⁻¹) and fed three diets (43% crude protein and 24% crude fat): a positive control (PC), a negative control (NC), and NC supplemented with 0.25% of a functional palatability enhancer (FPE) containing umami taste-stimulating substances. The PC contained 15% high quality FM and 5% soy protein concentrate (SPC), while the NC replaced 50% of FM and all SPC and wheat gluten with higher levels of less palatable and less digestible ingredients such as feathermeal hydrolysate, corn gluten meal, guar meal and sunflower meal. Growth performance, feed intake, feed conversion ratio (FCR), protein efficiency ratio (PER), and apparent digestibility coefficients (ADC) were evaluated. Additionally, expression of nutrient sensing and gut function markers (taste receptors and signalling effectors, enteroendocrine peptides, AA receptors and tight junction genes) was analysed in the foregut of 24h fasted fish at the end of the trial.

Results and Discussion

Reduction of FM in the NC diet negatively affected feed intake and growth performance compared to the PC. Supplementation with FPE significantly improved feed intake and growth (final body weight and specific growth rate, SGR), partially restoring these parameters (Figure 1A,B). Feed efficiency (FCR and PER) followed a similar trend, worsening in the NC and partly restored by FPE supplementation. Protein digestibility, but not energy or lipid digestibility, was significantly reduced in the NC compared to the PC, and significantly improved by supplementing the NC with FPE (Figure 1C).

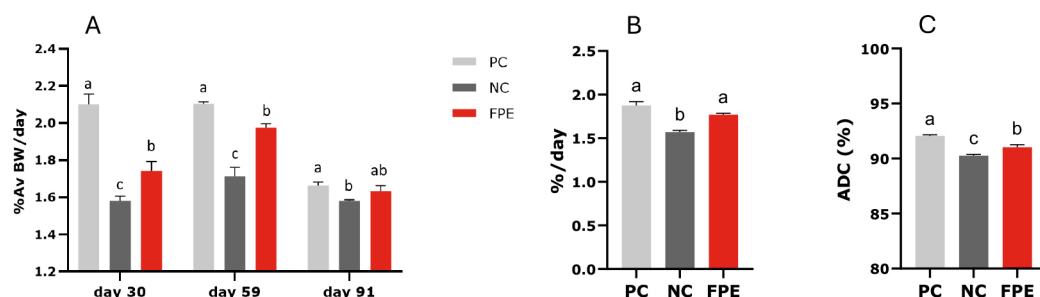


Figure 1. Performance indicators including feed intake along the experimental period (A), and specific growth rate (B) and apparent digestibility coefficient (ADC) of protein (C) at the end of the trial.

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MULTI-LAYER ANALYSIS TO CHARACTERIZE THE EFFECTS OF DIETARY FUCOIDAN FROM BROWN SEAWEED ON ATLANTIC SALMON EXPOSED TO INACTIVATED BACTERIA

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Introduction and Methodology

Feed additives derived from brown seaweed offer the potential to add value and unlock new market opportunities for algae cultivation. For instance, fucoidan from sugar kelp (*Saccharina latissima*) is a water-soluble sulfated polysaccharide that can be obtained through biorefinery processing (Michalak et al., 2023) for the development of novel functional aquafeeds (Rocha et al., 2025). Nevertheless, despite the reported intestinal immunomodulatory effects of fucoidan in salmonids (Rocha et al., 2025; Purushothaman et al., 2026), its comprehensive physiological impact on the host remains to be fully elucidated.

Currently, several methodologies are available to evaluate the properties of bioactive compounds in fish. For example, ‘omics’ strategies—such as transcriptomics, proteomics, and metabolomics—provide a general approach that can be complemented by the detection of specific indicators linked to both local and systemic physiological mechanisms. However, integrating and interpreting these miscellaneous datasets remains a significant challenge, yet this is essential for a overall characterization of the host’s response. Addressing this issue through multi-layer analysis provides an effective framework for correlating diverse methodological outcomes. To assess this approach, we performed a Data Integration Analysis for Biomarker discovery using Latent cOmponents (DIABLO). DIABLO is an n-block sparse Partial Least Squares Discriminant Analysis (sPLS-DA). Accordingly, we integrated results on intestinal microbiota composition, proteomic profiles, and specific physiological parameters of Atlantic salmon (*Salmo salar*). These fish were fed fucoidan-supplemented diets for four weeks in freshwater, transferred to seawater, and subsequently exposed to inactivated *Tenacibaculum maritimum*, an emerging bacterial skin pathogen.

Results and Discussion

Based on DIABLO, the distal intestine (DI) of fish fed fucoidan-supplemented diets during the freshwater stage featured *Lactiplantibacillus*—a proposed probiotic in Atlantic salmon (Rocha et al., 2023)—as a prominent member of the microbiota. Furthermore, a cluster representing the expression of *tnfa*, *cath2*, and *il8* (genes linked to innate immune activation) positively correlated with proteins involved in oxidative stress tolerance (B5XGZ2 and A0A1S3NRE4); mucosal barrier integrity (A0A1S3QBD3 and A0A1S3NMD0); and vesicular trafficking (A0A1S3T4X9). Interestingly, these indicators also showed a positive correlation with Enhancer of polycomb homolog 1 (A0A1S3NC89), a key protein linked to epigenetic regulation. Thus, these results suggested an activation/control of immune-related responses in DI. Similarly, DIABLO identified the gene expression of *tnfa* and *cath2* as relevant features in the head kidney (HK), clustering together with *anxa1* (a gene linked to immune homeostasis). Additionally, these biomarkers were associated with proteins involved in signaling and stress responses (B5XAP5); homeostasis and cellular maintenance (A0A1S3RWH8, A0A1S3RIW8, and B5X3X4); regulation of the actin cytoskeleton (B5X8M6 and A0A1S3R2S6); genomic, transcriptional, and epigenetic regulation (e.g., A0A1S3PGN9, B5X283, A0A1S3Q1N9, A0A1S3MUL1, B9ELF2, A0A1S3L589, B9EMR0); as well as energy demand (A0A1S3QT38). Therefore, the dietary inclusion of fucoidan would not only induce a local intestinal effect, but might also modulate the response in a major immune organ such as the HK. Regarding fish exposed to inactivated bacteria, the HK from fucoidan group showed the most relevant features after 24 hours. For instance, DIABLO detected a cluster of biomarkers connected with the expression of cytokines (*il6*, *il4/13*) and effector molecules, including antimicrobial peptides (*cath2* and *hepcidin*) and *arg1*. These results suggest the activation and balance of M1 and M2 macrophage subpopulations, which were linked to proteins involved in transcriptional regulation (B5X9L2 and B9ELV7), cellular signaling and homeostasis (A0A1S3LJM0 and A0A1S3M624), physical organization and structural integrity (A0A1S3NL71 and B9ELR2), and energetic demand (B5XB07).

Overall, the multi-layered DIABLO framework overcomes the limitations of single-dataset evaluations, allowing for a deeper characterization of the host response. Furthermore, this holistic approach provides robust evidence that dietary fucoidan exerts local and systemic immunomodulatory effects.

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Acknowledgment

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EARLY-LIFE MICROBIAL CONDITIONING SHAPES MICROBIOME STABILITY AND RESILIENCE IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

The microbiome is increasingly recognised as a key determinant of fish health [1], [2]; however, its functional role in aquaculture species remains poorly resolved, limiting translation into practice [3]. In this study, we examined the effect of early-life microbial conditioning on microbiome development and stability and fish performance under stress later in life. Germ-free rainbow trout (*Oncorhynchus mykiss*) eyed stage (~200 degree-days post-fertilisation) eggs were exposed to one of three microbial environments: (1) clean water with low microbial abundance, (2) water representative of conventional recirculating aquaculture systems (RAS), and (3) water inoculated with faeces from healthy adult rainbow trout. Hatched fish were reared under these conditions through the early developmental stages and subsequently subjected to chronic stress (e.g. high stocking density) at the juvenile stage (~5–10 g). Microbiome diversity, succession, and stability was characterised using Oxford Nanopore sequencing of the 16S rRNA gene across key developmental milestones. Host responses were quantified through growth performance, survival, and welfare indicators, alongside continuous monitoring of water quality and system microbial dynamics.

Results & Discussion

This work will establish whether targeted manipulation of early-life microbial environments can shape microbiome stability and enhance host resilience to chronic stress. The findings are expected to inform the development of early-life microbial interventions to improve robustness, reduce disease risk, and support sustainable intensification of aquaculture systems.

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MACROALGAE-DERIVED MULTIBIOTIC SUPPLEMENTATION IN GILTHEAD SEABREAM: PHYSIOLOGICAL AND WATER QUALITY RESPONSE TO A MODERATE HYPOXIA CHALLENGE

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Introduction

In modern aquaculture, the substitution of fish meals by vegetable-based diets shows compatibility in carnivorous species, but may produce physiological alterations that can be modulated by nutraceutical compounds derived from macro- and microalgae (Perera et al., 2020). At the same time, rising temperatures and high stocking densities reduce dissolved oxygen availability, which can trigger internal changes leading to physiological stress (Martos-Sitcha et al., 2020). Stress also affects the intestinal microbiota, which can be modulated through symbiotic nutraceuticals to provide beneficial properties to the individual (Egerton et al., 2018). Gut enzyme–bacteria interactions are also key for digestibility and protein utilisation, which influence ammonia, nitrate and nitrite release to the environment (Hlodzi et al., 2020). In this context, the present study evaluated the physiological response and water-quality changes in *Sparus aurata* fed with a macroalgae-derived multibiotic supplement under normoxic and moderate-hypoxia conditions.

Materials and Methods

The experiment was carried out in Servicios Centrales de Investigación en Cultivos Marinos (SCI-CM, Universidad de Cádiz, Spain). A total of 180 gilthead seabream (*Sparus aurata*) juveniles (54.58 ± 0.05 g average body mass) were distributed into 9 tanks (300 L, $n=20$ fish per tank), where three experimental groups were constituted to be fed with: i) a control diet (CTRL), or the same formulation supplemented with a ii) 1% and iii) 3.5% with the nutraceutical compound *Prebido II* produced by *Biotechnology Biopolym S.A.* (Granada, Spain). Feeding was performed *ad libitum* by twice daily meals for 91 days. Feed intake was controlled weekly and biometric samplings were performed every 4 weeks. After the feeding period, a hypoxia challenge was performed on the 3 experimental groups, being subjected to moderate hypoxia (35–40% O₂ saturation) during 24 hours. Biological samples allowed analysis of plasma, muscle and hepatic metabolites (glucose, glycogen lactate, triglycerides, cholesterol and proteins) and plasma cortisol. In addition, water samples were taken for water quality analysis (ammonium and nitrite concentration).

Table 1. Results of Feed Efficiency (A), Mesenteric Index (B) and Intestine Length Index (C). Results expressed as mean $\pm$ SEM, and analysed by one-way ANOVA analysis. Different letters represent statistically significant differences between groups (p -value <0.05).

Metabolite/Group	CTRL	B 1%	B 3.5%	p -value
FE (%)	0.71 ± 0.02^a	0.76 ± 0.02^{ab}	0.78 ± 0.01^b	0.041
MSI (%)	1.08 ± 0.09^a	0.82 ± 0.09^{ab}	0.58 ± 0.06^b	<0.001
ILI (%)	97.89 ± 4.00^a	81.05 ± 4.38^b	83.89 ± 4.80^b	0.014

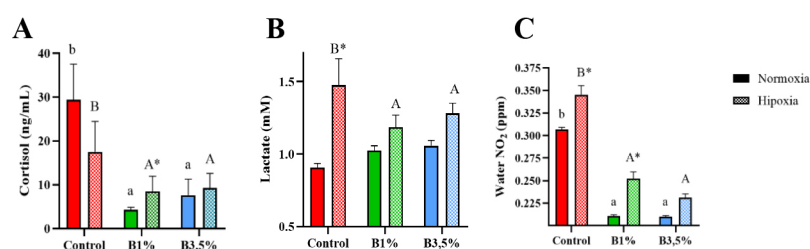


Figure 1. Plasma cortisol (A), lactate (B) and nitrite in water (C). Results expressed as mean $\pm$ SEM, and analysed by two-way ANOVA analysis. Different letters represent statistically significant differences between groups (p -value <0.05).

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Results and Discussion

In general, an effect on the parameters can be seen in relation to the *Prebiodo II* supplementation dose. Plasma cortisol levels showed a decrease in the supplemented groups both during normoxic and hypoxic conditions, while a lower lactate level was observed in these groups during a mid-hypoxia process, promoting a clear shift between aerobic and anaerobic metabolism, especially in those fish fed with supplemented diets (Fig. 1A, 1B). An inverse pattern was observed in hepatic glucose and glycogen values, responding to glucose mobilisation to maintain homeostasis. The good circulation, transport and energy use could be corroborated with the other parameters taken, showing an improvement in performance and metabolic status as a consequence of better nutritional absorption and water quality (Table 1, Fig. 1C). Overall, dietary supplementation modulated the physiological response to moderate hypoxia, improved feed efficiency and reduced mesenteric fat deposition, supporting the potential application of macroalgae-derived multibiotic compounds during stress-prone production phases in gilthead seabream.

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LEVERAGING ANNOTATION-FREE PSEUDO-LABELS FOR FISH MONITORING WITH TOP-DOWN POSE ESTIMATION APPROACH

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Introduction

Monitoring fish activity in aquaculture and marine conservation is essential for evaluating animal welfare and optimizing production. Accurate tracking of fish posture provides quantitative indicators of stress and environmental adaptation. However, deploying reliable computer vision systems in underwater environments remains challenging due to the scarcity of annotated data. Manual annotation of underwater footage is labor-intensive and prone to human bias, making large-scale dataset construction impractical.

While deep learning has advanced in terrestrial pose estimation, underwater settings introduce domain-specific obstacles, such as optical distortion, backscatter, and complex 3D movement. A critical technical challenge is the reliable determination of body orientation; visual similarities between head and tail regions, compounded by poor visibility and partial occlusions, often lead to incorrect anterior-posterior assignment.

This work proposes a multi-stage, annotation-efficient pipeline that bridges the gap between unsupervised geometric approximations and supervised deep learning. Building upon the annotation-free framework by Cebrián et al. (2025), we introduce a transfer learning strategy using curated pseudo-labels to fine-tune a ConvNeXt-based (Liu et al., 2022) pose estimator. Our primary contribution is the systematic resolution of orientation ambiguity through a “human-in-the-loop” refinement process, enabling robust behavioral monitoring with minimal manual labeling effort.

Methodology

The methodology follows a multi-stage pipeline, transitioning from an annotation-free approach to a minimally supervised transfer learning strategy:

Stage 1: Pseudo-label Generation. An initial annotation-free pipeline (Cebrián et al., 2025) employs GroundingDINO and the Segment Anything Model (SAM) for zero-shot detection and segmentation. Keypoints are localized using classical image processing (contour analysis and morphological operations). While efficient, this stage is prone to systematic head/tail flips because classical heuristics struggle to distinguish the snout from the caudal region under variable underwater lighting.

Stage 2: Curation and Transfer Learning. To rectify these limitations, a refined subset of 100 images from a dataset of 4280 pseudo-labels is selected through a manual quality filtering. During curation, frames with orientation inversions (identifiable by anatomical inconsistency) are either corrected or removed depending on the reliability of their pseudo-labels: frames with high-quality pseudo-labels are manually adjusted to fix errors, whereas those with poor-quality are discarded to reduce manual annotation effort. This refined subset is used to train a deep learning model within the SLEAP framework (Pereira et al., 2022). We adopt a top-down architecture for pose estimation composed of two ConvNeXt-based models: the first stage (top) localizes instance centroids, and the second stage (down) predicts keypoints. Both models are initialized with weights pre-trained on the ImageNet dataset (Deng et al., 2009) and fine-tuned on the curated aquatic pseudo-labels.

Table 1. Quantitative analysis of F1-Score, mPCK, TH, and mOKS relative to image count.

Nº images	Annotation-free	10	20	30	40	50	60	70	80	90
F1-Score	N/A	0.54	0.94	0.96	0.96	0.96	0.97	0.96	0.96	0.97
mPCK_T H	0.20	0.00	0.37	0.47	0.43	0.47	0.48	0.50	0.49	0.49
mOKS	0.21	0.09	0.33	0.41	0.42	0.41	0.44	0.47	0.44	0.45

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To evaluate this pipeline, a progressive training strategy was designed to analyze performance as a function of the amount of curated data. From the refined subset of 100 images, 10 were held out as an independent test set, while the remaining 90 images were used exclusively for training. These 90 images were incrementally partitioned into subsets of increasing size (10, 20, 30, ..., up to 90 images), and a separate model was trained for each subset under identical conditions. This iterative scheme enables a systematic assessment of how model performance scales with the availability of annotated data, providing insights into data efficiency and the marginal benefit of additional training samples within the proposed minimally supervised pipeline.

Results and discussion

The pipeline was evaluated on underwater sequences of luderick (*Girella tricuspidata*) and Australian bream (*Acanthopagrus australis*) (Ditria et al., 2021), using Mean Average Precision (mAP) at an Object Keypoint Similarity (OKS) threshold of 0.5 (mOKS), F1-score for visibility (annotation-free approach is excluded from visibility metrics since it forces all keypoint detections regardless of occlusion), and the average of Percentage of Correct Keypoints (PCK) only focusing on head and tail targets (mPCK_TH) to assess pose and orientation performance. Table 1 reports strong performance with small training sets and clear improvements over the annotation-free baseline. The smallest training set (10 images) does not exceed the annotation-free baseline, whereas performance improves and stabilizes from ~30 images onward, indicating an efficient trade-off between annotation effort and accuracy. The proposed method corrects systematic orientation errors in annotation-free approaches by learning discriminative visual features that capture head–tail asymmetries, improving the reliability of behavioral metrics.

Conclusions

This work presents a multi-stage pipeline for fish pose estimation bridging annotation-free and supervised learning, enabling orientation-consistent keypoint detection with minimal annotation effort. The method improves accuracy over annotation-free heuristics by correcting systematic orientation errors through discriminative visual features capturing head–tail asymmetries. Future work will delve to iteratively expand training dataset and self-supervised learning to improve generalization and leverage unannotated data.

Acknowledgements

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A FUNDAMENTAL MODEL FOR OXYGEN CONSUMPTION OF ATLANTIC SALMON

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Introduction

Predicting oxygen availability in Atlantic salmon farms is challenging, but digital simulations that couple bioenergetics and hydrodynamics show great promise. Robust simulations depend on reliable estimates of oxygen demand, yet previous empirical models offer limited accuracy. Here, we present a refined fundamental model for Atlantic salmon oxygen consumption rate (MO_2) as a function of three readily measurable parameters: body weight, water temperature, and relative swimming speed (Morin et al., 2026).

Materials and Methods

Retaining the established framework of Grøttum and Sigholt (1998), we refined the model through an improved coefficient estimation approach and a methodologically rigorous dataset derived from group swim tunnel respirometry measurements on 718 fish across seven experiments. Model coefficients were re-estimated using log-linear regression fitted via nonlinear mixed-effects, substantially improving parameterisation and yielding a model that explains 80% of the observed variation in MO_2 .

Figure 1

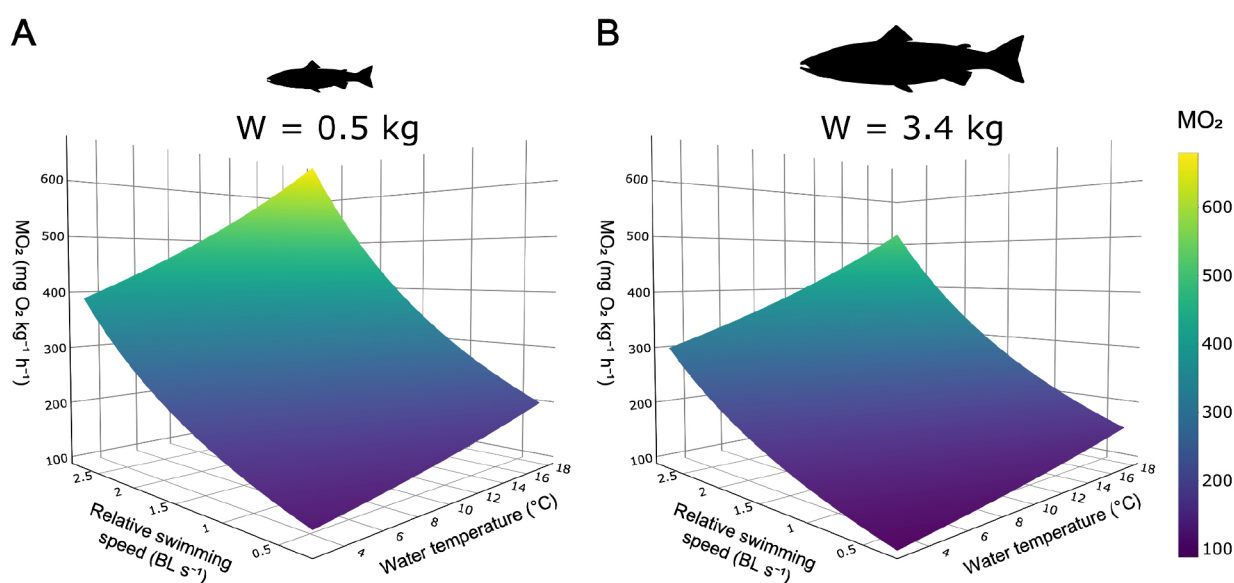


Fig. 5: Three-dimensional surface contours illustrating oxygen consumption rate (MO_2 ; $mg\ O_2\ kg^{-1}\ h^{-1}$; z-axis) as a function of relative swimming speed (U ; body lengths $[BL]\ s^{-1}$; y-axis) and water temperature (T ; $^{\circ}C$; x-axis). Results are displayed for two body weights (W ; kg): (A) 0.5 kg and (B) 3.4 kg , selected as the most representative weights of our refined dataset. Surface contours are generated from our model developed using log-linear regression fitted via nonlinear mixed-effects: $MO_2 = 79.7W^{-0.14}1.04T^{1.63}U$.

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Results

The log-linear regression fitted via nonlinear mixed-effects showed the strongest performance, with adjusted $R^2 = 0.80$ and root mean square error (RMSE) = 56.39, outperforming the model by Grøttum and Sigholt (1998) which had an adjusted $R^2 = 0.68$ and RMSE = 71.50. Our model is as follows:

$MO_2 = 79.7W^{0.14}1.04^T1.63^U$, where MO_2 is oxygen consumption rate ($\text{mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$), W is body weight (kg), T is water temperature ($^{\circ}\text{C}$), and U is relative swimming speed (body lengths s^{-1}).

Discussion

Our refined fundamental model for oxygen consumption of Atlantic salmon represents a substantial advance over previous empirical models. It spans an extensive range of metabolic rates ($60\text{--}700 \text{ mg O}_2 \text{ kg}^{-1} \text{ h}^{-1}$), fish sizes ($0.2\text{--}3.38 \text{ kg}$), water temperatures ($3\text{--}18 \text{ }^{\circ}\text{C}$), and relative swimming speeds ($0.31\text{--}2.84 \text{ BL s}^{-1}$), capturing the diversity of conditions in modern salmon aquaculture. This core formulation defines baseline MO_2 for Atlantic salmon and serves as a foundational component within digital simulation frameworks. With broad utility in both research and industry, our model supports the development of more precise, data-driven strategies for modern salmon aquaculture.

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BEYOND PHOSPHORUS DIGESTIBILITY: INORGANIC FEED PHOSPHATE INFLUENCES ABSORPTION OF OTHER MINERALS IN ATLANTIC SALMON (*Salmo salar*)

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Introduction

Phosphorus (P) is an essential nutrient for fish, playing key roles in energy metabolism through ATP synthesis, skeletal mineralization, cell membrane structure, nucleic acid synthesis, and cellular signalling (Skonberg et al., 1997) and to observe the magnitude of these changes over time. Fish with an initial mean weight of 1.8 g were hand-fed one of five semipurified wheat-gluten based diets containing between 0.23%–1.16% dietary phosphorus, or a fish meal control diet (1.8% P. As most P present in plant-derived ingredients is bound to phytate and remains poorly digestible for fish (Nelson et al., 1968) or poorly utilized by, the chick. In addition, phytic acid chelates mineral elements, including calcium, and thereby reduces their availability in whole or part. However, the quantitative effect of phytic acid on the total dietary level of any element in a feed has been neglected. It is important, therefore, to know the phytic acid content of feeds in order that adequate amounts of essential elements may be supplied, particularly phosphorus and calcium. Common (1940, dietary supplementation with inorganic feed phosphates (IFP) is often required to meet nutritional requirements. However, P discharge from aquaculture is a major contributor to eutrophication of aquatic ecosystems (Luo, 2022). Therefore, identifying highly digestible P sources is critical to improve P utilization efficiency while minimizing environmental losses. The present study evaluated the apparent digestibility of four IFPs in freshwater Atlantic salmon (*Salmo salar*): monosodium phosphate (MSP), monoammonium phosphate (MAP), monocalcium phosphate (MCP), and calcium humophosphate (CHP). The objective was to identify the most digestible P sources and assess their effects on the digestibility of other nutrients and minerals.

Materials and Methods

Five experimental diets were formulated. A control diet (CTRL) was produced without IFP supplementation and contained 2% diatomaceous earth as an inert filler. Four additional diets were formulated by supplementing the control diet with MCP (0.71%), MAP (0.62%), MSP (0.68%), or CHP (0.74%). Inclusion levels were adjusted to provide equivalent total P across all supplemented diets. All IFPs were incorporated before extrusion, replacing equivalent amounts of diatomaceous earth. Yttrium oxide (0.02%) was included in all diets as an inert digestibility marker. The experimental diets were tested in quadruplicate groups of 25 Atlantic salmon (initial body weight: 55 ± 3 g) reared in 350-L tanks supplied with freshwater at $12.7 \pm 0.3^\circ\text{C}$. Following a 32-day feeding period, faeces were collected by stripping for digestibility analyses.

Results

No significant differences were observed among treatments for growth performance parameters, including weight gain and feed conversion ratio (FCR). Survival was 100% throughout the trial. P digestibility was significantly influenced by the phosphate source. MSP (96%), MAP (93%), and CHP (87%) exhibited significantly higher P apparent digestibility coefficients than MCP (71%). Although CHP achieved higher P digestibility than MCP (+ 15 % units), MSP and MAP displays higher P digestibility than CHP. The phosphate source also affected the digestibility of several other minerals including zinc, magnesium, sodium and calcium. Calcium digestibility was the highest in fish fed with the CHP diet (~34%), significantly exceeding values observed with MCP, MAP, and MSP (~26–28%). This effect may be related to Ca-complexing properties of CHP as previously observed in poultry (Coudert et al., 2025). Protein and energy digestibility coefficients were modestly but significantly higher in MSP and MAP-supplemented diets compared with MCP and CHP-supplemented diets.

Discussion

This study provides valuable insights, as comparative digestibility data among multiple inorganic phosphate sources in Atlantic salmon remain scarce in the literature. Among the evaluated IFPs, MSP and MAP provided the highest P digestibility in freshwater Atlantic salmon. Interestingly, the ranking and values of P digestibility observed among the different IFPs in Atlantic salmon closely matched those reported in rainbow trout (Morales et al., 2018), suggesting a consistent utilization pattern across salmonid species.

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MSP displays an improvement of about 25 % units in P digestibility compared to MCP, highlighting its potential to substantially reduce P discharge from salmon farming systems as well as nitrogen compared to MAP. Although CHP demonstrated lower P digestibility than MAP and MSP, it outperformed MCP and substantially enhanced P digestibility by 15 % units. These findings suggest that CHP may have potential applications in species fed diets containing less digestible plant-based ingredients, as its complexing properties may enhance mineral availability.

Furthermore, the phosphorus source significantly influenced the digestibility of sodium, magnesium, zinc, and selenium, potentially enabling a reduction in their dietary inclusion levels. This aligns with recommendations from regulatory authorities, including the European Food Safety Authority (EFSA), which has proposed lower zinc supplementation levels in salmon feeds.

Overall, the results highlight that IFPs influence markedly P digestibility but also the absorption of several other minerals. Consequently, IFP selection should not be considered only as a strategy to meet dietary P requirements, but also as a nutritional lever influencing the digestibility of macro- and trace minerals.

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COMPARTMENT-SPECIFIC MICROBIOTA RESPONSES TO CYCLIC TEMPERATURE AND SALINITY CHANGES IN GILTHEAD SEA BREAM

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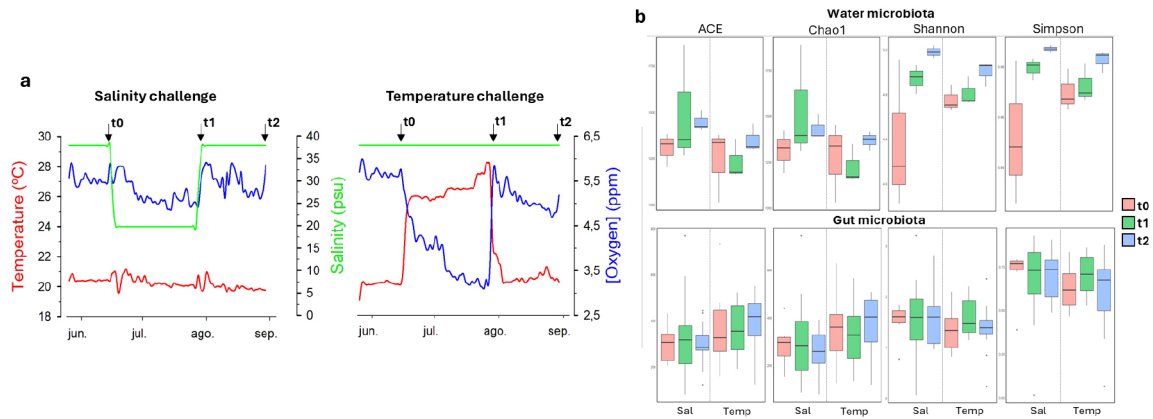
Introduction

Aquaculture is one of the fastest-growing food production sectors worldwide, yet its long-term sustainability is threatened by climate change. This challenge is particularly relevant in the Mediterranean basin, where its semi-enclosed nature amplifies warming trends which include salinity and oxygen fluctuations (Dayan et al., 2023). Aquatic organisms respond to environmental stress through a complex set of physiological, cellular, and molecular mechanisms. Within this framework, the microbiota emerges as a potential marker of stress response and as a key component of the host’s adaptive capacity (Domingo-Bretón et al., 2025). In fact, the gut, skin, and surrounding water harbor interconnected microbial communities that, along with the host, form the holobiont, an integrated biological unit which reacts in a coordinated manner to environmental change. Despite this importance, relatively limited and sometimes contradictory information is available on how changes in temperature and salinity reshape microbiota in farmed species. Accordingly, the present study aimed to characterize gilthead sea bream microbiota changes in the intestinal tract, skin, and rearing water in response to shifts in temperature and salinity. Understanding these dynamics is essential to identify whether specific environmental perturbations induce transient dysbiosis, functional reorganization, or recovery to the original state in the sea bream holobiont system.

Materials and methods

A total of 120 gilthead sea bream (*Sparus aurata*) juveniles (~13.4g) were randomly distributed into six 90-L tanks (20 fish/ tank) connected to two independent RAS. Three tanks were allocated to the salinity challenge and three tanks to the temperature challenge, performing a closed-loop trials, characterized by a decrease in salinity and an increase in temperature from as illustrated in Figure 1a. Before the start of the trial, fish were acclimated for two weeks under common conditions (20 °C, seawater, natural photoperiod, DO> 75%) and were fed manually to visual satiation with a commercial diet. The experimental program included three sampling points (t0, t1, t2), used to collect skin, intestinal and farm water microbiota samples. Microbiota samples were processed for DNA extraction and sequenced using Oxford Nanopore Technologies (16S rRNA regions V1-V9) for skin and water samples, and Illumina MiSeq (16S rRNA region V3-V4) for gut microbiota samples. For both platforms, sequences identified as ASVs were taxonomical assigned using Minimap2 v2.17-r941 with SILVA v138.1 as reference database. Temporal and between-branch differences were assessed using alpha and beta diversity metrics. In addition, partial least squares discriminant analysis (PLS-DA) and Bayesian network analysis was applied to identify bacterial clusters associated with environmental changes, whereas MaAsLin2 was used to model longitudinal microbial trajectories and associations across time and experimental conditions.

Figure 1. Experimental design (a). Alpha diversity metrics for water and gut microbiota (b).



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Results and discussion

The results indicate that the water microbiota responded more strongly to environmental perturbations than the gut microbiota, both under temperature-driven and salinity-driven changes. In particular, alpha diversity exhibited a temporal patterns in water samples, whereas gut samples showed a more homogeneous profile across sampling times or experimental branches (Figure 1b). This trend was further supported by beta diversity and discriminant analyses, which consistently identified environmental changes as the main driver of shifts in the water microbiota. In contrast, the intestinal compartment appeared to be more stable and buffered, likely reflecting the role of the host in maintaining gut homeostasis, as previously reported (Naya-Català et al., 2026). At the taxon level, LEfSe and MaAsLin2 identified multiple bacterial markers associated with environmental change in the rearing water, including taxa that responded similarly, as common stress-related signature, regardless of the perturbation. Further analyses are ongoing to evaluate whether analogous patterns extend to the skin microbiota and to a more integrative, with the aim of defining the broader physiological response of the holobiont to environmental stress. Overall, these findings support the view that the water microbiota acts as a highly responsive environmental sensor, whereas the gut microbiota behaves as a more constrained and host-filtered compartment, thereby contributing to the maintenance of holobiont stability under fluctuating conditions.

Funding

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A BAYESIAN MULTI-OMICS STRATEGY TO INVESTIGATE HOST–MICROBIOTA DYNAMICS IN AQUACULTURE

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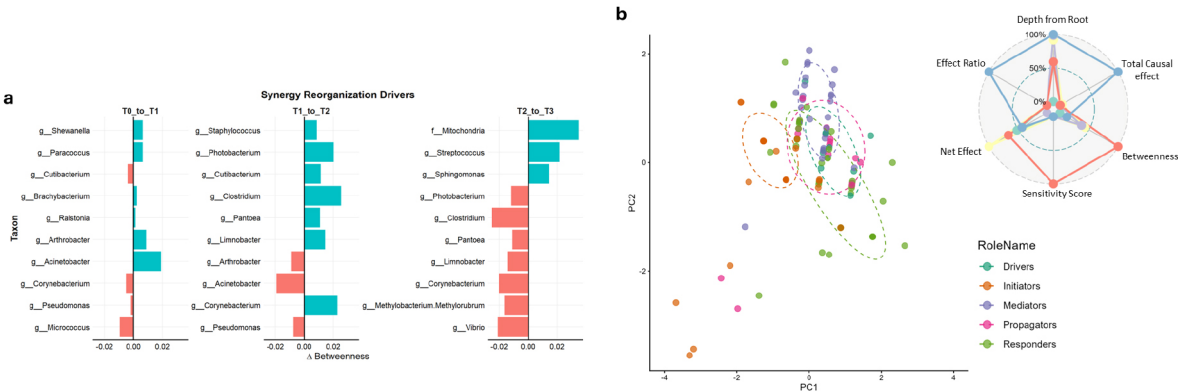
Introduction

Modern aquaculture increasingly requires analytical frameworks capable of capturing the complexity of host responses to environmental and nutritional challenges. Isolated measurements often provide only partial insight, whereas integrative approaches can reveal the coordinated behavior of host physiology, microbial communities, and environmental drivers (Ruiz-Perez et al., 2021). The study of microbiota in aquaculture has traditionally focused on describing taxonomic composition, often across different body compartments such as gut, skin, gills, and the surrounding water. However, these datasets are rarely interpreted as parts of a connected biological system. Therefore, integrating microbial profiles from distinct host-associated niches and linking them with host-centered omics, such as transcriptomics, creates the opportunity to move from descriptive ecology toward mechanistic interpretation. This systems-level perspective is especially relevant in aquaculture, where performance traits, welfare, disease resistance, and environmental tolerance are shaped by multiple interacting biological layers. Among the available approaches, Bayesian networks (BN) are particularly attractive because they allow the inference of probabilistic relationships and within complex biological systems and support inferential hypothesis generation (Moroni et al., 2025). Their application in aquaculture could therefore provide a powerful route to uncover the host-microbe cross-talk and to support the development of predictive and function-based models of animal response.

Materials and methods

This study adopted an integrative multi-experiment approach. For this purpose, different trials involving dietary inclusion of functional ingredients, environmental contaminants, and alternative feed formulations were considered. Microbiota datasets derived from different compartments (gut, skin, water) and where available, they were further combined with additional omics layers (host gene expression data). The base of this study was the integration with the Bayesian network inferences, which were performed using the SAMBA, a computational platform developed by our research group (Soriano et al., 2023). This approach allowed the reconstruction of probabilistic causal networks describing dependencies between microbial features and omics data. Network structures were then processed using a customized R-based pipeline composed by a suite of network metrics, including degree centrality, weighted connectivity, betweenness, closeness, and hierarchical properties such as ancestor–descendant relationships and node depth. The strength and direction of inferred causal relationships were extracted and analysed from the conditional probability tables (CPTs), associated with each node. All these values were then integrated into matrices to compute total causal effects, capturing both direct and indirect propagation of influence across the network. Based on these outputs, nodes were classified into functional roles (e.g., initiators, drivers, propagators, mediators, and responders), reflecting their behaviour, position, and influence within the microbial system.

Figure 1. Important taxa ranked by Betweenness (a). PCA and radar plot of the roles within BN (b).



(Continued on next page)

Results and discussion

The results obtained so far demonstrate the potential of this pipeline to integrate 16S rRNA gene-based microbiota datasets and multi-omics data through Bayesian network modeling. This approach made it possible to explore the potentially causal relationships within the microbial communities, such as those established between fish skin and surrounding water, across time. In particular, the analysis highlighted how bacterial taxa shared by both compartments change over time and how their ecological roles within the community may shift in response to the experimental stimulus (Figure 1a). At the multi-omics level, the downstream integration of metataxonomic and gene expression data enabled the characterization of ecological roles using both topological and non-topological metrics from BN, leading to the identification of mixed clusters composed of bacterial taxa and host genes, which responded to the experimental trial (Figure 1b). These findings represent a valuable starting point for a more detailed functional characterization and provide new insight into the processes underlying host-microbiota cross-talk. Future developments of this pipeline should further increase the level of omics integration, including the identification of key receptors potentially involved in host-bacterial interactions and the incorporation of metabolomic profiles to complement and strengthen the interpretation of these mechanisms. In the long term, this framework may contribute to a better understanding the details of how animals respond to experimental perturbations and may support the development of mitigation strategies, combining microbiome- and host-based solutions, aimed at improving welfare and productivity in aquaculture under different scenarios.

Funding

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DEVELOPMENT OF A HIGH-DENSITY SNP ARRAY FOR A MAJOR AQUACULTURE SPECIES, THE PACIFIC OYSTER *Crassostrea gigas*

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Introduction

The Pacific oyster (*Crassostrea gigas*) is a major aquaculture species in Europe and worldwide, yet its production is increasingly threatened by recurrent disease outbreaks, environmental fluctuations, and the impacts of climate change. In this context, genomic tools represent a powerful avenue to accelerate selective breeding and enhance the resilience of farmed populations. However, the development and implementation of such tools in molluscan species remain particularly challenging.

Bivalve genomes are characterized by exceptionally high levels of polymorphism, structural variation, and complex genomic architecture, which complicate both variant discovery and genotyping assay design. This high genetic diversity can lead to probe hybridization issues, allelic dropout, and poor clustering during genotype calling, ultimately reducing genotyping accuracy. As a result, existing single nucleotide polymorphism (SNP) arrays and genotyping platforms developed for *C. gigas* have shown limited performance, with genotype call rates sometimes as low as 25%, thereby constraining their utility for downstream applications such as genomic selection (Jourdan et al. 2023) and GWAS.

Despite these limitations, genomic selection programs have already been initiated in *C. gigas* (Boudry et al. 2021), highlighting both the urgency and the potential impact of improved genomic tools. There is therefore a critical need to develop a robust, high-density, and well-optimized genotyping tool that can overcome these species-specific constraints and deliver high-quality, reproducible genotype data suitable for routine use in breeding programs.

In this study, we report the development of a high-density SNP genotyping array specifically designed for *C. gigas*, with particular emphasis on maximizing marker informativeness, genome coverage, and genotyping performance. By testing this HD array on a wide range of diverse population, we will produce a consensus, good quality SNP list to design routine, cost-effective and efficient genotyping tools.

Materials and Methods

A total of 199 Pacific oysters from diverse genetic backgrounds were used, including individuals from disease resistant and susceptible lines, as well as samples from IFREMER datasets from different locations. Whole-genome sequencing (WGS) was performed using the Illumina HiSeq 2500 platform (150 bp paired-end reads). Variant discovery was conducted using the Genome Analysis Toolkit (GATK), followed by stringent filtering criteria including minimum depth (DP ≥ 5), genotype quality (GQ ≥ 30), minor allele count (MAC ≥ 5), and removal of indels and multi-allelic variants. Approximately 10.12 million high-quality SNPs were retained across the genome.

SNP selection for the array design was based on multiple criteria: minor allele frequency (MAF), genome-wide distribution, coverage of telomeric regions, linkage disequilibrium, and functional annotation. Additional filtering incorporated Affymetrix recommendations, prioritizing SNPs with high probe performance and minimizing A/T and G/C polymorphisms due to increased probe space requirements. A high-density SNP array was designed using a 6-micron format, targeting approximately 480K markers.

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Resulting array was tested on 5 populations (60-96 samples/population) from 5 breeding programs from IFREMER and the biggest oyster hatcheries in Europe (Vendée Naissain, MARINOVE, SATMAR, Novostréa) to evaluate the informativeness, number of SNP working on each population, and effectiveness in real cases of pertinent potential usage.

Results

Whole-genome sequencing identified over 113 million SNPs and ~33 million indels across the genome, corresponding to approximately 138 million total variants. After filtering, ~10.12 million high-quality SNPs were retained, with an average density of ~18,000 SNPs per Mb. Mutation analysis showed a transition/transversion (Ti/Tv) ratio of approximately 1.23, indicating good data quality and expected mutation patterns. SNPs were distributed across coding and non-coding regions, with the majority located in intergenic and intronic regions. The final high-density array included 482,279 SNP markers, with balanced genome-wide coverage and a median inter-marker distance of ~540 bp, compared to ~100 bp in the full WGS dataset. The selected SNPs showed appropriate MAF distribution and improved uniformity across chromosomes. Results from genotyping the 5 populations included in the project showed a 98% sample call rate, a 52.6% SNP call-rate, (254 306 SNPs classified as “PolyHighResolution”). Average MAF for those SNPs is 0.31.

Discussion

This study demonstrates that the development of a high-density SNP array for *Crassostrea gigas* was feasible despite the well-known challenges associated with highly polymorphic molluscan genomes. The combination of diverse whole-genome sequencing data and stringent SNP selection criteria resulted in a marker panel with good genome-wide coverage and high informativeness. The array showed strong performance in terms of sample call rate (98%), confirming its robustness across diverse populations. Although the SNP call rate (52.6%) remains moderate, it represents a clear improvement over previously reported tools and still provides a large set of high-quality polymorphic markers (>250K) with high MAF, suitable for most genomic applications.

The variability in SNP performance across populations highlights the importance of including diverse genetic backgrounds during array design, particularly for aquaculture species with heterogeneous breeding stocks. Testing the resulting tool on those diverse background appears very necessary to validate the pertinence of the new tool.

This array therefore represents a valuable tool for genomic selection, GWAS, population genomics, and the management of genetic diversity in oysters. Overall, while some technical limitations persist, this work constitutes a significant step toward the routine implementation of efficient and cost-effective genomic tools in oyster breeding programs.

Acknowledgment

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POPULATION-SPECIFIC RESPONSES OF *Ostrea edulis* TO ESTUARINE VARIABILITY: IMPLICATIONS FOR AQUACULTURE AND RESTORATION

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Introduction: Natural oyster reefs, once dominant in estuaries worldwide, have undergone severe global decline, with ~85% loss¹. In Europe, the native species *Ostrea edulis* is now considered threatened due to overexploitation, habitat loss and disease outbreaks², which has led to restoration and reintroduction efforts supported by policy initiatives and aquaculture development. However, these efforts face increasing challenges from climate change, particularly shifts in temperature and salinity regimes, alongside pollution and pathogen pressure, all of which affect growth, physiology, and survival of the oysters. This *in situ* field study evaluates survival and growth of *O. edulis* under natural estuarine variability, assessing population-specific responses and tolerance to low salinity and other environmental stressors to support the selection of resilient stocks for aquaculture and restoration programs.

Material and methods: Juvenile oysters from two distinct origins, Mediterranean Sea (Sardinia, Italy) and North Sea (Koster Islands, Sweden), were deployed in February 2025 at a commercial oyster farm in Lima estuary (NW Portugal), characterized by strong salinity (~3-30 PSU) and temperature variations (~10-19°C). Monthly assessments included survival, growth, pathogen prevalence (e.g., *Bonamia ostreae*, *Marteilia refringens*, *Ostreid herpesvirus-1*, *Perkinsus* spp), while microplastic (MPs) contamination was evaluated before deployment (T0) and at 1, 3, 6, and 12 months after deployment. Environmental conditions were continuously monitored to detect environmental stress events like flooding and heatwaves.

Results: Survival differed significantly between origins ($p < 0.0001$), with Italian oysters showing higher survival than Swedish oysters throughout most of the study (> 75% vs ~42% after 10 months).

Complete mortality of both stocks occurred after 12 consecutive days of near-zero salinity caused by severe storms (Figure1). Growth also differed significantly ($p < 0.001$), with higher absolute growth rates in Italian oysters (0.03 mm day⁻¹) than in Swedish oysters (0.003 mm day⁻¹). MPs contamination varied between origins and sampling periods, with higher loads in Italian oysters (7.44 MPs ind⁻¹ and 59.50 MPs g⁻¹) than Swedish oysters (5.45 MPs ind⁻¹ and 5.14 MPs g⁻¹). Italian oysters showed a contamination peak in March (T1) relative to pre-deployment levels (T0), followed by a decline, whereas Swedish oysters maintained consistently low MPs levels throughout the study. Fibers were the dominant MPs type in both stocks, mainly colourless, blue, black, and grey. Although MPs concentrations in water varied over time, no clear correlation was observed between MPs concentrations in water and oyster tissues. Regarding pathological survey, no evidence of *Marteilia refringens*, *Bonamia ostreae*, OsHV-1, or *Perkinsus* spp. was detected.

Discussion and conclusion: Differences in survival and growth between origins suggest population-level variation in the response of *O. edulis* to estuarine conditions, with contrasting acclimation dynamics between Italian and Swedish oysters. Growth patterns indicate origin-specific strategies, with higher absolute growth in Italian oysters. Putative MPs contamination was detected in all samples, with higher levels in Italian oysters. As the confirmation of polymer composition by FTIR spectroscopy is still in progress, these particles are classified as putative MPs. The lack of direct correspondence between MPs concentration in water and oysters suggests that MPs accumulation is influenced by factors other than ambient water concentrations. The non-detection of major oyster pathogens suggests low pathogen pressure during the study period and supports the potential suitability of the Lima estuary for *O. edulis* cultivation and restoration initiatives. Overall, this study provides preliminary evidence of population-specific differences in oyster performance and susceptibility to environmental stressors. These findings will help identify the most suitable stock for aquaculture and ecological restoration programmes in Portuguese estuarine systems.

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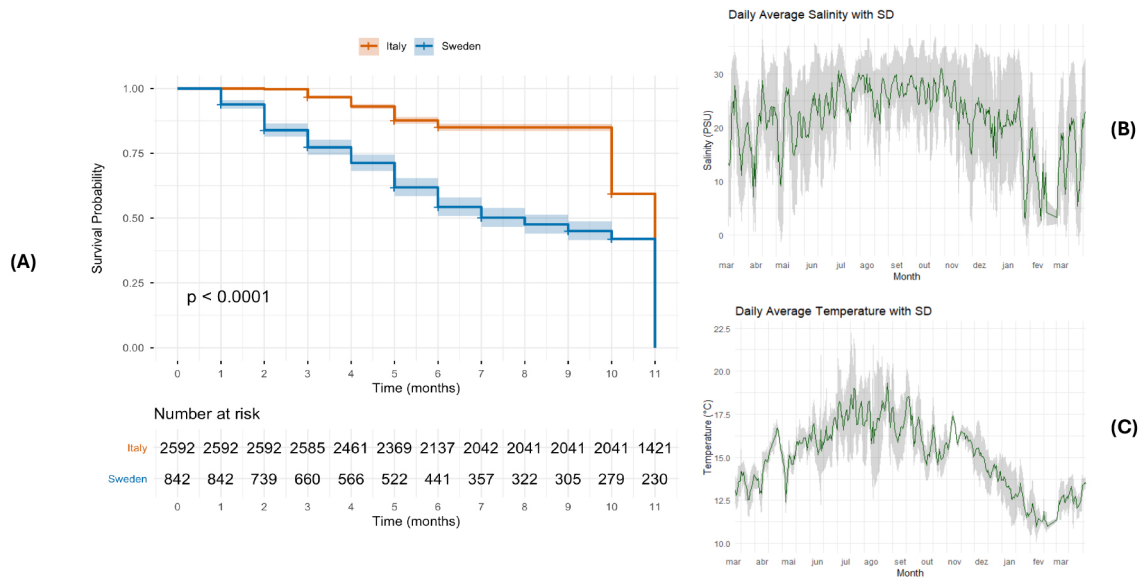


Figure 1. (A) Kaplan-Meier survival curves comparing oysters of Italian and Swedish origins over 11 months; Average daily salinity **(B)** and temperature **(C)** in the estuary over 12-month period, expressed as average $\pm$ SD.

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LIPID MEDIATORS OF INFLAMMATION RESOLUTION IN GILTHEAD SEABREAM (*Sparus aurata*): EFFECTS OF INSECT OIL INCLUSION UNDER ACUTE STRESS

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Introduction and Methodology

Inflammation is a natural biological process that eliminates harmful stimuli and promotes tissue repair. Under normal conditions, inflammation resolution is actively mediated by specialized pro-resolving lipid mediators (SPMs) derived from the n-3 long-chain polyunsaturated fatty acids, EPA and DHA. However, disruption of this process under stressful conditions may impair immune homeostasis and compromise health. While SPMs have been studied in mammals, their role in teleost fish remains poorly understood. In aquaculture, the increasing use of alternative lipid sources, such as insect oils, raises questions about their impact on lipid-mediated pathways involved in stress and inflammation, particularly under routine practices such as confinement and handling. The present study evaluated the response of juvenile gilthead seabream (*Sparus aurata*) to an acute stress event after 56 days of feeding with one of two experimental diets: a control diet containing fish oil and poultry oil, or a BSFLO diet in which *Hermetia illucens* oil fully replaced poultry oil (5.2% inclusion). Fish were then subjected to 1 min of air exposure followed by confinement. Blood samples were collected at the end of the feeding trial (before stress exposure) and 90 min after stress exposure to quantify resolvin-E1 and maresin-1 in plasma. These plasma SPMs were measured by ELISA (n=9) and analysed by two-way ANOVA (diet and stress as fixed factors).

Results

Acute stress significantly reduced plasma resolvin-E1 levels ($p = 0.009$), with higher values in non-stressed fish regardless of diet, while maresin-1 remained unchanged. Diet did not affect resolvin-E1, but BSFLO-fed fish tended to show higher maresin-1 levels ($p = 0.069$). These findings suggest a rapid utilization of resolvin-E1 during the early phase of inflammation resolution, whereas maresin-1 may contribute to later stages of the process. Further research is required to better elucidate the temporal dynamics of inflammation resolution in gilthead seabream.

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INDUCING POLYPLOIDY IN *Ulva fenestrata* VIA ANTIMITOTIC TREATMENT AND VALIDATION BY DNA QUANTIFICATION

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Introduction

Total DNA quantification in seaweeds is paramount important and it enables selective breeding and physiological understanding. Measuring DNA content identifies genetic quality and potential in candidate organisms for aquaculture development, supporting the selection of strains with faster growth, higher nutrient profiles, or enhanced stress tolerance. Since DNA content reflects cell ploidy levels the number of chromosome sets quantification reveals how ploidy influences seaweed growth rates, stress responses, metabolic efficiency, and environmental adaptation. Seaweed of the genus *Ulva* widely known as sea lettuce, is a high protein content and fast-growing plant and can grow in wide range of climate. *Ulva fenestrata* stands out as a premier candidate for sustainable aquaculture, notable for offering exceptionally high levels of bioactive vitamin B12, balanced essential amino acids, and efficient nutrient bioremediation and serves as a versatile feedstock for functional foods, animal feed additives, and eco-friendly biomaterials.

Materials and Methods

The aim of the present investigation was to develop polyploidy lines in *U. fenestrata* with desired features like sterile plant, robust and resilient feature, enhanced growth rate and high nutrient content. In order to achieve it, the germilings of *U. fenestrata* was treated with different concentrations of anti-mitotic agent colchicine (0, 100, 500, 1000 and 2000ppm) and incubated under different temperature conditions (5°C, 10°C and 15°C but with same photon intensity (100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) for a period a one month and assessed the total DNA content was was quantified using NanoDrop (Thermo Fisher Scientific, NanoDropTM), Qubit (Thermo Fisher Scientific, Qubit 4 Fluorometer) and gene-specific quantitative PCR (qPCR) for more precision.

Results

The results show that *U. fenestrata* responded well to oryzalin and found to increase the DNA content (Fig 1). Total DNA Germilings treated with 500ppm at incubated at 10 and 15 degree was found significantly ($p < 0.05$), therefore, 3X of control plant suggesting that it responded well to oryzalin and could be used for generating triploid sterile plants. The DNA extracted were found to be high quality in terms of purity index (A260/A280) with a range of 1.78 – 2.53 (average 1.98 ± 0.20). Plants incubated in different temperature responded differently to the antimitotic agent. At 5°C, increase in DNA content was not statistically significant despite there were marginal increase in DNA content over control plants at 500ppm of colchicine (1.23) and and 2000pp (1.27), these represent roughly 20-27% increase in DNA content relative to control but they are not statistically significant. Plant treated at 10°C and 15°C responded well to colchicine, at 10°C, 100ppm with dramatic DNA elevation ratio (3.70), representing a 270% increase suggesting temperature affects colchicine sensitivity. Similar observations were recorded with plants incubated under 15°C i.e. DNA elevation ratio of 3.36, 5.90, 3.86 and 3.58 were recorded in plants treated with 100, 500, 1000 and 2000ppm respectively. The results obtained from nanodrop methods were further validated by qubit. Overall results suggest that antimitotic treatment caused polyploidy line in seaweed *U. fenestrata*, probably triploid plant (ratio more than 3). However, further studies required to validate the results through assessing the nuclear DNA content by flowcytometry to precisely represent the chromosomes level in the treated cells against control plant cell and systematic karyotype studie (chromosome numbers) as well to investigate development of polyploidy (triploid, and or tetraploidy) in *U. fenestrata*.

Conclusion

Present results show that seaweed *U. fenestrata* responded well to the anti-mitotic agent colchicine, However, studied on the impact of other antimitotics that are commonly used in land crop breeding like oryzalin and trifluralin can be tried and incubated under different light regime (red, green and blue) with different photon density to optimize the induction method and develop potent polyploidy lines for bolstering aquaculture production.

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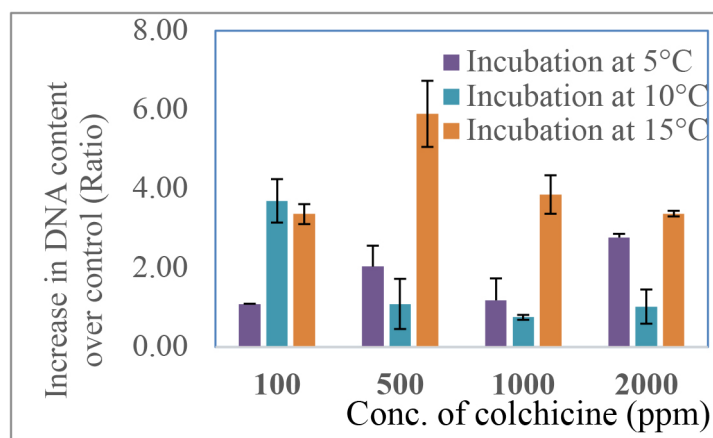


Fig 1: Increase in DNA content of seaweed *Ulva fenestrata* treated with anti-mitotic agents over control plants (results expressed in 'ratio')

Acknowledgment

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KARYOTYPE ANALYSIS AND CHROMOSOME CHARACTERIZATION OF *Saccharina latissima*: TOWARDS POLYPLOIDY STRAIN DEVELOPMENT

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Introduction

The sugar kelp *Saccharina latissima* has received intense scientific attention over the last decades due to their great potential to be utilized as human food, biomolecules feed and feed additives. In Norway as well as in other coastal European countries, the commercial-scale farming of the sister species *S. latissima* has been widely successfully demonstrated within the past decade. Norway has nutrient rich seawaters, diverse seaweed biodiversity and conducive geographical structure which can favor aquaculture of this species at commercial scale. Development of Karyotyping and systematic analysis of an organism's chromosome composition is crucial for seaweed breeding programs because it provides essential genetic information that directly impacts crop improvement, selection efficiency, and biotechnological applications. Therefore, breeders can identify distinct cytotypes (organisms with different chromosome numbers) and use this knowledge to select genetically diverse parents for crosses, which increases the likelihood of producing plantlets with desirable agronomic traits including accelerated maturation, structural robustness, stress resilience, vigorous growth, and elevated nutrient accumulation.

The objective of the present study was to analysis the karyotype of *S. latissima* and establish its chromosome numbers. 1–2 mm of tissue was cut from young sporophyte and pre-treated with 0.04% 8-hydroxyquinoline for 4 hours at 24 °C, followed by fixation in ethanol:acetic acid (3:1) for 72 hours at 24 °C. Hydrolysis was performed using 0.25 N HCl for 10 min. at 24 °C, followed by enzymatic digestion in a mixture of cellulase, macerozyme and pectolyase (2:1:1) and alginate lyase (3 unit/ml) for 1.5 hours at 37 °C. Then chromosomes were spreaded onto glass slides using a drop of ethanol:acetic acid solution and allowed to air-dry. The prepared slides were stained with DAPI and examined under fluorescence microscopy at 1000× magnification (KERN) and analysed using ImageJ software. Parallely, gametophytes (male and female) and young sporophytes of *S. latissima* was subjected to antimitotic agents like colchicine, oryzalin, and trifluralin under different temperature and light regime to assess the polyploidy condition by checking the increase in chromosome numbers

Results

The chromosome number for *S. latissima* could not be fully established due to variation within samples and tissue itself. Over 300 microscope were slides prepared by following different procedures and examined them through 1000x magnification in oil immersion, but chromosome appeared clumsy and no clear image was obtained (Fig 1). Recent literature shows several different estimates in chromosome numbers in *S. latissima* depending on the methodology used.

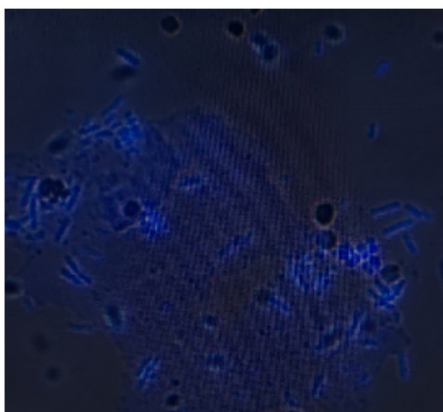


Fig 1: Image of chromosome of *S. latissima* observed under fluorescence microscopy at 1000× magnification (KERN)

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Flow cytometry studies by Phillips et al. (2011) estimated 62 chromosomes, while microscopy-based observations by Liu et al. (2012) and Liu et al. (2022) found 31 chromosomes. Genome assembly estimates from various recent reports (Cormier et al., 2017; Fan et al., 2020a; Shan et al., 2020; Diesel et al., 2023) range from 28–34 chromosomes. The latest genetic linkage mapping work on European *S. latissima* by Denoeud et al. (2024) identified 31 pseudochromosomes. The discrepancies arise because chromosome number predictions in brown macroalgae are complicated by sex-specific polyteny and population-specific ploidy variation in sugar kelp meristem sporophyte tissue. Different estimation methods flow cytometry, traditional microscopy, and modern genome assembly techniques can yield different results. The most recent and reliable estimate appears to be 31 chromosomes based on microscopy observations and the latest genome assembly with genetic linkage mapping (Denoeud et al., 2024).

Conclusion

The lack of consensus on the chromosome number in *S. latissima* reflects broader challenges in cytogenetics of brown macroalgae, where traditional and modern methods yield conflicting results. The range of estimates (31 to 62 chromosomes depending on methodology) suggests that no single approach has yet definitively resolved this question. High-resolution fluorescence in situ hybridization (FISH) using species-specific probes should be applied to multiple *S. latissima* populations and life stages. This would allow direct visualization and counting of individual chromosomes while accounting for any polyteny or somatic variation. Traditional Giemsa staining coupled with modern imaging systems could also provide clearer chromosome morphology and banding patterns for karyotyping.

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EFFECTS OF A HOST-DERIVED PROBIOTIC DIET ON REPRODUCTIVE PERFORMANCE AND MICROBIAL COMMUNITY COMPOSITION IN COMMON CARP (*Cyprinus carpio*)

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Introduction

Integrating selective breeding with microbiome management is widely proposed as a way to make aquaculture more sustainable by improving disease resistance, stress tolerance, and reducing chemical inputs (Lorgen-Ritchie et al., 2023). Despite this potential, the role of the host-associated microbiome in influencing gamete quality and fertilization success in fish remains insufficiently explored. Recent advances in species-specific probiotic feeding strategies suggest that microbial communities in the gut—as well as in the immediate environment of gametes—can contribute not only to enhanced growth performance, as widely documented (Amoah et al., 2023), but also to improved reproductive outcomes in fish (Enzeline et al., 2024). We aimed to develop a probiotic formulation based on a bacterial strain isolated from the carp gut with previously identified beneficial effects by our research group. This probiotic was subsequently evaluated in a recirculating aquaculture system in feeding trials using multiple concentrations.

Materials and Methods

Three experimental groups were established. The control group (C) received a standard compound feed without probiotic supplementation, while the two treatment groups were fed diets supplemented with bacteria at concentrations of 10^6 CFU g⁻¹ (T6) and 10^8 CFU g⁻¹ (T8), respectively. Sperm quality was assessed based on the following parameters: osmolality, pH, motility and kinetic parameters, concentration, and relative sperm count (spermatozoa per kilogram of body weight; spz bwkg⁻¹). Five males were examined per group. During propagation, pooled egg batches from females of the control group were used for fertilization. To evaluate the effect of males, fertilization and hatching rates were determined. Microbiological analyses included the determination of colony-forming units (CFUs) from sperm samples as well as gut samples of both males and females, using non-selective TSA and selective MRSA media. In addition, the composition of the gut microbial community was analyzed. Gut samples were collected from five males and five females from each experimental group, and microbial community structure was characterized using Illumina 16S rRNA gene (V3–V4 region) paired-end sequencing.

Results

No significant differences were found among the three groups (C, T6, T8) in sperm motility, pH, osmolality, or concentration and relative sperm count. Similarly, no significant differences were detected in fertilization rate. However, a significant difference was observed in hatching rates among the groups. Compared to the control group, the hatching rates of both the T6 and T8 groups were significantly higher. In sperm samples, the number of colony-forming units (CFUs) determined on TSA medium was significantly higher in the T8 group compared to the T6 group. No significant differences were observed between treatments on the selective MRS agar medium. The bacterial diversity in the digestive tract was similar between the control group and the 10^6 CFU g⁻¹ treatment group, whereas lower diversity values were observed in the 10^8 CFU g⁻¹ group. In most individuals, the gut microbiota was dominated by members of the phyla Fusobacteriota (3.5–92%), Pseudomonadota (1–34%), and Spirochaetota (0–63%). Within the Pseudomonadota phylum, sequences belonging to the genus *Aeromonas* were predominant in the control and 10^6 CFU g⁻¹ groups; however, their relative abundance decreased in fish fed the 10^8 CFU g⁻¹ bacteria-supplemented diet.

Discussion

Probiotic supplementation did not affect key sperm quality parameters or fertilization rate, indicating that basic reproductive functions remained unchanged. However, the significantly higher hatching rates in the treated groups suggest a beneficial effect at later developmental stages, possibly through improved microbial conditions or enhanced embryo viability. The increased bacterial counts in sperm samples and shifts in gut microbiota composition, particularly the reduced abundance of *Aeromonas* at higher probiotic doses, indicate that microbial community modulation may play a role in these outcomes. Although microbial diversity decreased in the highest dose group, this did not negatively affect performance. The decrease in *Aeromonas* abundance may be considered beneficial due to its potential role as an opportunistic pathogen. Overall, the results highlight the potential of probiotic application to improve reproductive success in aquaculture, mainly by enhancing hatching efficiency rather than altering sperm quality.

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Acknowledgment

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ENVIRONMENTAL SUSTAINABILITY OF OYSTER FARMING AND INFLUENCE OF CLIMATE CHANGE

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Introduction

Farmed bivalves are often considered low-impact products, and oyster aquaculture is increasingly discussed within the transition toward more sustainable and resilient blue food systems (Gephart et al., 2021). However, environmental performance of oyster farming remains strongly system- and site-dependent. In addition, existing Life Cycle Assessment (LCA) studies show highly heterogeneous results, partly driven by methodological variability, which limits systematic cross-study comparison (Vélez-Henao et al., 2021). This limitation is particularly relevant under climate change, where future conditions may increase warming-induced mortality (De Marco et al., 2023; Shi et al., 2024) and accelerate the degradation of exposed farming materials, affecting productivity and input requirements, and ultimately reshaping environmental impacts per unit of product (Jouannais et al., 2023; Niero et al., 2015). This study applies a harmonized cradle-to-gate LCA framework to four case studies of Pacific oyster production (*Magallana gigas*) across different environments and farming methods, including inshore longlines with lantern nets, intertidal off-bottom culture on trestles with mesh bags, and a lagoon suspended-rope system with tidal simulation. Beyond comparing baseline environmental performance and identifying system-specific hotspots, the study explicitly integrates climate-change scenarios into the LCA framework to assess how future seawater warming and reduced material lifetimes, under more frequent extreme events, may affect environmental performance. Specifically, the objectives are to: (i) quantify the environmental performance of the four production systems; (ii) identify the main hotspots within each case study; (iii) assess how climate-driven changes in mortality and infrastructure durability affect LCA results; and (iv) evaluate case-specific mitigation strategies.

Materials and methods

A cradle-to-gate LCA was conducted for four oyster farming systems located in Algeria (ALG), Scotland (DUR), and Italy (offshore-GIU and lagoon-SCA). System boundaries included seed supply (transport to the farm), grow-out, depuration (when present), and packaging. The functional unit was 1 kg of live packed oysters. Primary data were collected through farm surveys and site visits, while background processes were modelled using Ecoinvent 3. Impacts were assessed using ReCiPe 2016 Midpoint (H) and Cumulative Energy Demand. Effects of climate change was integrated into the LCA as two scenarios: (A) increased mortality due to seawater warming, applied to GIU case study for which a comprehensive dataset of climate data is available, and (B) reduced durability of infrastructure under more frequent extreme events. In scenario (A), historical seawater temperature data (2006–2024, CMEMS) were used to derive a smoothed seasonal climatology (May–September), while future projections for 2030 and 2050 (RCP 4.5) were obtained from the POLCOMS-ERSEM model. Heat stress was quantified using threshold exceedance metrics (26–30 °C) and species-specific tolerance limits from the literature. A semi-empirical model was then used to translate excess thermal exposure into mortality, assuming a non-linear response to temperature. Resulting mortality rates were expressed as a scaling factor (F), representing additional upstream inputs per functional unit, and applied to seed supply and grow-out phases. In scenario (B), a 30% reduction in the lifetime of grow-out materials was assumed to reflect increased exposure to extreme events. Uncertainty and sensitivity were assessed using Monte Carlo simulations and rank correlation analysis.

Results

Baseline LCA results confirmed relatively low environmental impacts, with Global Warming Potential ranging from 1.82 to 2.29 kg CO₂ eq per kg of oysters. However, substantial variability was observed across systems. The grow-out phase was the main contributor to impacts in all case studies, although key drivers differed, including electricity use in ALG, diesel consumption in GIU, material use in SCA, and a combination of inputs in DUR. Mitigation scenarios reduced several impacts, mainly through electrification and the integration of renewable energy. However, these improvements were accompanied by increases in some other categories (e.g., marine eutrophication and water consumption), indicating burden-shifting. Climate change scenarios consistently increased environmental impacts. Under Scenario (A), warming-induced mortality in the GIU system rose from 30% (baseline) to 32.8% (2030) and 52.2% (2050) on average, leading to GWP increases of +2–7% and +25–89%, respectively. Scenario (B) led to impact increases across all systems, with the largest effects observed on marine eutrophication (+28–40%) and water consumption (+6–28%), while other categories showed more moderate increases (<11%).

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Discussion and Conclusions

The results demonstrate that oyster aquaculture cannot be represented by a single environmental profile. Environmental performance is highly context-dependent, being shaped by system design, site conditions, and operational practices. Although oyster production is generally associated with low impacts, substantial variability emerges across systems, with similar life-cycle stages driving impacts but system-specific environmental drivers requiring tailored mitigation strategies. This variability has important implications for large-scale assessments and policy development, as generalized assumptions may lead to misleading conclusions. Improvements targeting energy use may lead to trade-offs in other impact categories, highlighting the need for multi-criteria assessment. The integration of climate scenarios shows that environmental performance is dynamic rather than static. Climate change can increase impacts through two main pathways: reduced biological efficiency (i.e., higher mortality) and increased material demand due to shorter infrastructure lifetime. These effects are non-linear and become more pronounced under long-term warming, altering the relative performance of farming systems. Overall, a scenario-based LCA emerges as a more robust and forward-looking framework for assessing aquaculture sustainability under changing environmental conditions. Future research should refine site-specific mortality modelling, expand the range of climate drivers, and integrate economic feasibility to enhance the applicability of results at larger scales.

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SITE-SPECIFIC CLIMATE ADAPTATION SCENARIOS FOR MEDITERRANEAN MUSSEL FARMING IN THE CENTRAL ADRIATIC SEA: BALANCING THERMAL-STRESS MITIGATION AND PRODUCTION SUSTAINABILITY

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Introduction

Marine heatwaves are increasing in frequency, intensity, and spatial extent across the Mediterranean Sea, with documented impacts on bivalves (Galli et al., 2017; Masanja et al., 2023). The Mediterranean mussel (*Mytilus galloprovincialis*) is the main farmed bivalve in the Mediterranean region and a strategic sustainable blue food (EUMOFA, 2022; Gephart et al., 2021). Extreme warming has caused mass mortality of mussels in the Adriatic Sea, with reported losses approaching 80% during summer 2024. Adaptation strategies in mussel aquaculture must be site-specific because biological growth is driven by local environmental gradients (e.g., temperature, food supply) and profitability is dependent on logistics and market demands. Indeed, effective adaptation must balance both ecological and economic feasibility. This study evaluates site-specific adaptation scenarios in Abruzzo region (Italy) by integrating thermal stress reduction and growth performance.

Materials and Methods

Five sites along a coast-offshore transect (5, 7.5, 10, 15, and 20 km from shore) were combined with two farming depths ("surface" and "deep") to define ten adaptation scenarios (Fig. 1). Daily seawater temperature, chlorophyll-a, and dissolved oxygen for 2006–2025 were obtained from CMEMS and averaged across each farming layer. These variables forced an individual Dynamic Energy Budget model for *M. galloprovincialis* (Sarà et al., 2012), scaled to farm production through cohort-based simulations accounting for mortality, reproduction, harvesting, size structure, and meat content. Model calibration incorporated local farmer information and production reports. Production cycles initiated between 2006 and 2023 were simulated and compared annually with the 5-km surface baseline (current farm location). Thermal stress above 28 °C (Anestis et al., 2007) was quantified by its frequency, duration, and intensity, while production performance was evaluated from biomass yield, growth, harvest timing, and meat content. Non-inferiority criteria identified adaptation scenarios reducing thermal exposure without economically relevant production losses.

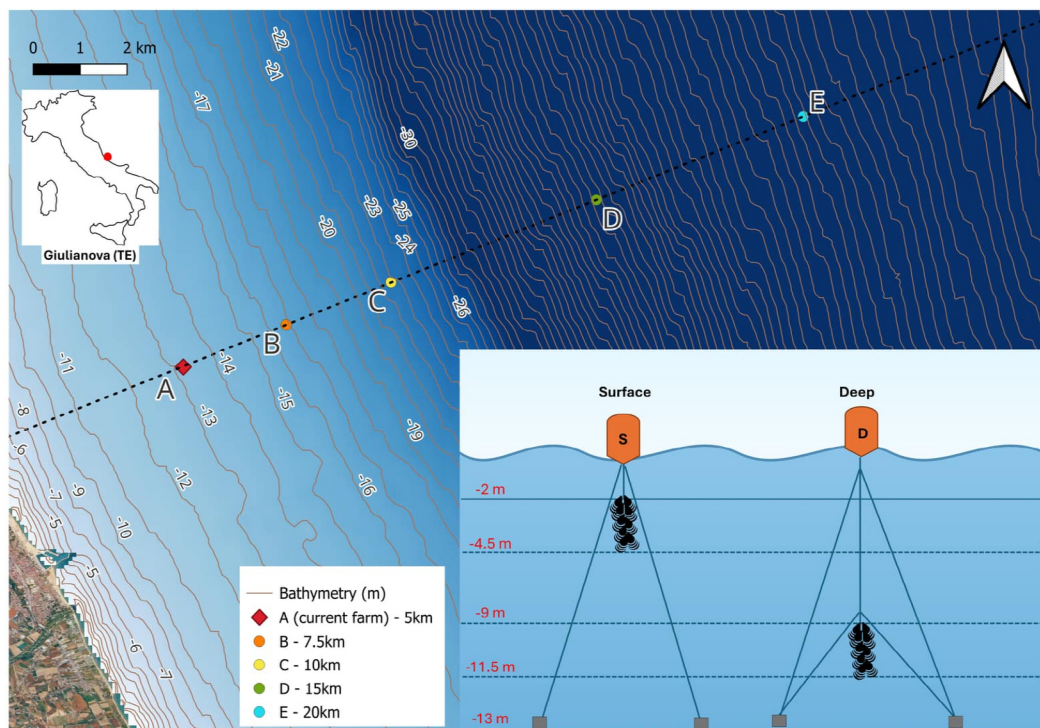


Figure 1. Spatial and vertical configuration of the ten adaptation scenarios. Five sites located 5–20 km from the coast were combined with “surface” and “deep” farming layers.

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Results and Discussion

Results showed a consistent trade-off between thermal stress mitigation and production performance. Both long-line submersion and offshore relocation reduced thermal exposure, with benefits increasing along the coast–offshore gradient and peaking when the two strategies were combined. However, stronger adaptation interventions progressively reduced productive performance. Scenarios closer to the current farm largely maintained baseline conditions, whereas deeper and more offshore configurations produced substantial production losses. Thus, the scenarios providing the greatest thermal protection were not compatible with current commercial requirements. These findings show that effective climate adaptation in mussel aquaculture cannot be based solely on thermal-stress reduction but requires a site-specific, multi-criteria assessment integrating biological performance, operational feasibility, and economic viability.

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SPIRULINA SUPPLEMENTATION IN FRESHWATER FISH: A COMPREHENSIVE REVIEW OF REPRODUCTIVE EFFECTS

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Introduction

Arthrospira platensis (spirulina) a filamentous cyanobacterium commonly used as a functional feed ingredient in aquaculture, is characterized by a high nutritional value, including 60–70% protein content (dry biomass), essential amino acids, polyunsaturated fatty acids, vitamins, minerals, and bioactive pigments. Numerous studies have demonstrated the beneficial effects of spirulina supplementation on fish growth performance, immune responses, antioxidant capacity, and reproductive traits, highlighting its potential as a sustainable and protein-rich alternative to conventional aquafeed ingredients [1,2].

Understanding the molecular mechanisms underlying the reproductive effects of spirulina is important for optimizing nutritional strategies in aquaculture. Therefore, the aim of this study was to investigate the effects of dietary spirulina supplementation on fish reproduction through a comprehensive literature review, to develop a curated database and perform in silico functional analyses to identify candidate genes and molecular pathways involved in reproductive regulation. The identified molecular targets will further support the interpretation of an ongoing nutritional trial in European grayling (*Thymallus thymallus*), a salmonid species of high conservation interest in Europe [3].

Materials and Methods

A comprehensive literature review was conducted to identify studies investigating the effects of Spirulina supplementation and its bioactive compounds on reproductive performance and gamete quality in freshwater fish. Based on the identified evidence, candidate genes and molecular pathways potentially involved in Spirulina-mediated reproductive responses were selected for functional analyses. Protein–protein interaction networks and functional enrichment analyses were performed using the STRING database.

Results

Literature analysis revealed that spirulina supplementation may positively influence multiple aspects of fish reproduction. In males, reported effects included improvements in sperm volume, motility, viability, and density. In females, spirulina supplementation was associated with increased gonadosomatic index, fecundity, spawning frequency, egg quality, and egg size. Positive effects were also reported for fertilization success, hatching performance, larval quality, and survival. Furthermore, several studies indicated enhanced yolk deposition and stimulation of vitellogenesis. Functional analyses identified candidate genes and molecular pathways potentially involved in reproductive regulation and responses to spirulina supplementation.

Discussion

The available literature highlights spirulina as a promising functional feed ingredient for supporting reproductive performance in freshwater fish. The reported improvements in sperm quality, fecundity, egg quality, fertilization success and vitellogenesis suggest that Spirulina-derived nutrients and bioactive compounds may influence key reproductive processes through complex nutritional and molecular mechanisms. The integration of literature-based evidence with functional genomics and molecular docking approaches provides new insights into potential pathways underlying these effects. Identification of candidate genes and regulatory networks offers a framework for understanding how dietary spirulina may modulate reproductive physiology.

These findings provide a foundation for evaluating Spirulina supplementation in European grayling, where optimization of reproductive efficiency is relevant for both sustainable hatchery production and conservation-oriented aquaculture. The ongoing nutritional trial will further determine whether the molecular responses predicted through in silico analyses are reflected in reproductive performance, physiological traits, and gene expression patterns.

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BIOLOGICAL RESPONSE OF FISH MODELS TO TRIFLUOROACETIC ACID: FROM CELL LINES TO EARLY DEVELOPMENTAL STAGES

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Introduction

Trifluoroacetic acid (TFA) is an ultra-short-chain per- and polyfluoroalkyl substance (PFAS) and the final degradation product of several fluorinated compounds. Due to its extreme persistence and continuous environmental release, TFA concentrations are increasing worldwide in aquatic ecosystems, including surface waters, groundwater, and drinking water sources (Neuwalde et al., 2022). Although TFA has historically been considered less hazardous than long-chain PFAS, concerns regarding its long-term ecological effects have recently intensified due to its irreversible environmental accumulation and increasing global distribution (Arp et al., 2024). Current risk assessments largely focus on acute toxicity endpoints and conclude that environmental concentrations remain below known toxicity thresholds. However, such assessments often overlook chronic exposure scenarios, episodic contamination events, and subtle physiological or molecular effects that may occur prior to the onset of overt toxicity. Therefore, further investigation is required to determine whether environmentally occurring TFA concentrations can affect aquatic organisms at different biological levels, and thus aquatic ecosystems and production systems. The present study aimed to evaluate the effects of TFA exposure using complementary *in vitro* and *in vivo* fish models, including fish cell lines and zebrafish (*Danio rerio*) embryos and larvae.

Materials and Methods

Three fish cell lines (EPC, SAF-1 and RTG-2) were used to assess the cellular effects of TFA exposure. Medium acidification because of TFA addition was monitored. Cytotoxicity was evaluated for different concentrations of TFA at 24 h of exposure. Also, antibacterial activity was also tested in different pathogenic species with relevance in aquaculture. Functional assays based on viral infection susceptibility were performed in EPC cells following a pre-treatment of the cells with TFA. In addition, transcriptomic analyses (RNA-Seq) were conducted on cells exposed to 2 µM TFA, a concentration representative of environmentally relevant exposure scenarios. To investigate the effects of TFA during early fish development, zebrafish embryos were exposed to different concentrations of TFA and AA. Embryo survival, larval survival and hatching rates were monitored throughout development according to standard zebrafish embryo toxicity protocols. The use of AA controls in each experiment allowed differentiation between specific TFA effects and those potentially associated with medium acidification.

Results

TFA exposure did not significantly alter the culture medium pH at concentrations up to 128-256 µM, depending on medium composition. No acute cytotoxicity was detected in EPC, SAF-2 or RTG-2 cells, even at concentrations up to 1024 µM. Pre-treatment with TFA did not modify cellular susceptibility to VHSV or SVCV infection in EPC cells. Similarly, no antibacterial activity was detected at the concentrations tested. In zebrafish assays, exposure to TFA was associated with lower survival in embryos and larvae compared with control and AA-treated groups. On the other hand, exposure to TFA did not affect hatching success in zebrafish embryos. Final hatching rates were comparable across all treatments and controls, and no consistent alteration in hatching dynamics was observed following TFA exposure. However, preliminary transcriptomic analyses revealed significant gene expression changes largely affecting diverse biological functions.

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Discussion

The absence of acute cytotoxicity in fish cell lines (and aquatic bacteria) supports previous studies suggesting relatively low acute toxicity of TFA compared with other PFAS. Furthermore, the lack of detectable effects on viral susceptibility indicates that essential innate immune functions remain largely unaffected under the exposure conditions tested. Similarly, the zebrafish embryo assays indicated that exposure to TFA did not affect hatching success. Although a tendency towards lower embryo and larval survival was observed in TFA-exposed groups. Further analyses are required to determine the biological relevance of the observed survival differences. Moreover, the absence of visible developmental toxicity does not preclude the occurrence of subtle molecular or physiological alterations. The ongoing transcriptomic analyses will contribute to a better understanding of potential sublethal responses and help improve future environmental risk assessment of this increasingly prevalent contaminant.

Acknowledgment

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SPAWNING INDUCTION IN AZOREAN LIMPETS (*Patella aspera* AND *Patella gomesii*): CHEMICAL AND PHYSICAL STIMULI

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Introduction

Overexploitation driven by the high economic and cultural value of patellid limpets across the Macaronesian archipelagos, including the Azores, has severely depleted wild populations (Castejón *et al.*, 2022). As a result, Azorean limpets (*Patella aspera* and *Patella gomesii*) represent promising candidates for aquaculture, offering a sustainable path to relieve pressure on natural stocks. However, controlled captive reproduction remains a critical bottleneck (Castejón *et al.*, 2022). While physical stimuli, such as thermal shock and vigorous aeration, can induce gamete emission in several *Patella* species, variable success rates hinder protocol standardization (De Girolamo *et al.*, 2024; Nascimento *et al.*, 2025). Concurrently, neuroendocrine regulation in limpets is poorly understood, though exogenous neuropeptides have triggered spawning in related gastropods like *Haliotis asinina*, *Cellana sandwicensis*, and *Patella ferruginea* (Ferranti *et al.*, 2021; Nhan & Ako *et al.*, 2019; Nurai *et al.*, 2010). To address these constraints, this study evaluates both chemical and physical spawning induction pathways in Azorean limpets. This work investigates the first application of hormonal treatments (OvaRH, aELH, and Pu-GnRH) alongside the optimization of non-invasive physical cues to induce gamete release in these species. By assessing spawning success, latency, and gamete output, this research offers a practical framework to support future standardized and reproducible spawning protocols for Azorean limpets.

Material and methods

Wild-harvested *P. aspera* and *P. gomesii* broodstock were evaluated under two spawning induction approaches: (1) hormonal and (2) physical stimulation. The broodstock was acclimatized for at least 2 weeks and the baseline gonadosomatic index was determined (). To evaluate hormonal cues, limpets were individually housed in 300 mL boxes () in a flow-through system and assigned to four injection treatments: control (saline), OvaRH (sGnRHa), aELH (*Aplysia* egg-laying hormone), or Pu-GnRH (*Patella ulyssiponensis* GnRH) (per species). Each specimen received a single intramuscular injection of , delivering a target dose of . Individual injection volumes were calculated using species-specific shell length (SL)-soft body weight (BW) regressions (*P. aspera*: ; *P. gomesii*:). Spawning occurrence, latency, and gamete output were monitored over 72 h. The second approach evaluated established physical cues combined with a novel photoperiod factor (continuous darkness) in *P. gomesii* (). Animals were exposed for 5 h across four treatments (): darkness without intense aeration at 18°C (control); darkness with intense aeration; darkness with intense aeration and cold shock (); darkness with intense aeration and warm shock (). Spawning occurrence, latency, gamete output, and terminal GSI were quantified.

$(GSI \% = \frac{Gonad\ weight}{Total\ soft\ body\ weight} \cdot 100)$. To evaluate hormonal cues, limpets were

Results

Hormonal treatments (OvaRH, aELH, and Pu-GnRH) failed to induce spawning in *P. aspera*, with no gamete release events recorded across any treatment group. Similarly, hormonal induction was largely ineffective in *P. gomesii*, yielding a single gamete release event in the OvaRH treatment (3% success rate). Despite relatively high mean baseline values of 14% for *P. aspera* and 27% for *P. gomesii* (mature broodstock), the administered hormonal dose () failed to trigger spawning. Post-treatment mortality in *P. aspera* was substantial across all hormonal treatments (OvaRH: 20%; aELH: 30%; Pu-GnRH: 13%) and highest in the saline control group (43%). Mortality in *P. gomesii* was lower but remained notable in certain groups (OvaRH: 13%; aELH: 0%; Pu-GnRH: 17%; control: 20%). In contrast, the physical stimulation approach proved highly effective in *P. gomesii*. The highest spawning success was achieved in the darkness combined with intense aeration and warm thermal shock treatment (92%), followed by darkness with intense aeration (58%), darkness with intense aeration and cold thermal shock (50%), and the darkness-only control (33%). Spawning latency was short, with most gamete release occurring during treatment exposure (81% for male and 100% for female spawners). Total gamete output was highest in the darkness combined with intense aeration and warm thermal shock, which produced the greatest total counts for both sperm () and oocytes (). The mean terminal of non-spawning specimens evaluated at the end of the physical trial was 23% (mature).

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Discussion

Overall, this study highlights a clear contrast in spawning induction efficacy for Azorean limpets. The application of exogenous hormones failed to reliably trigger gamete release while causing substantial injection-related mortality. Conversely, non-invasive physical cues, particularly darkness combined with intense aeration and warm thermal shock, achieved a 92% spawning success rate and the highest gamete yields recorded. Although these findings significantly advance our understanding of spawning dynamics in Azorean limpets, further investigation is needed to evaluate protocol reproducibility and assess downstream gamete quality.

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SAND-BASED ENVIRONMENTAL ENRICHMENT AS A STRATEGY TO IMPROVE GROWTH PERFORMANCE AND PHYSIOLOGICAL RESPONSES IN FLATHEAD GREY MULLET *Mugil cephalus*

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Introduction

In recent years, environmental enrichment (EE) has attracted increasing attention in the field of animal science as a key strategy to improve animal welfare. Although environmental enrichment has been widely investigated in terrestrial species, its application in aquaculture remains relatively limited (Ojelade et al., 2022). 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 sand-based environmental enrichment could enhance growth performance along with related physiological responses in flathead grey mullet is crucial for improving aquaculture sustainability.

Materials and Methods

An experimental diet was formulated to contain low fishmeal (3%) and soybean meal (10%) levels. Diet 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). 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), feed conversion rate (FCR) and serum biochemistry were calculated. Differences among treatments were considered significant at $p < 0.05$ (Student's t-test).

Figure 1

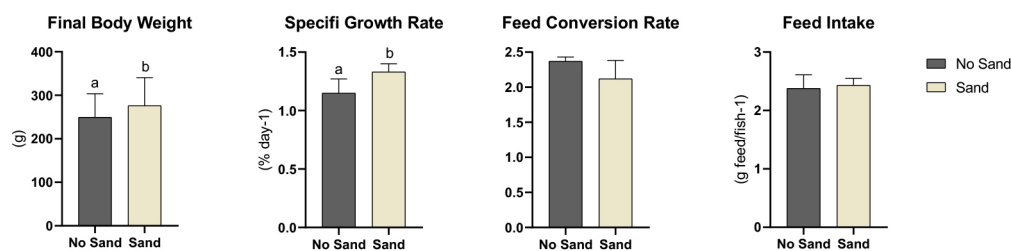


Figure 1. Final body weight, specific growth rate, feed conversion rate and feed intake of flathead grey mullet at two environmental enrichments (with/without sand). Different letters indicate significant difference.

Figure 2

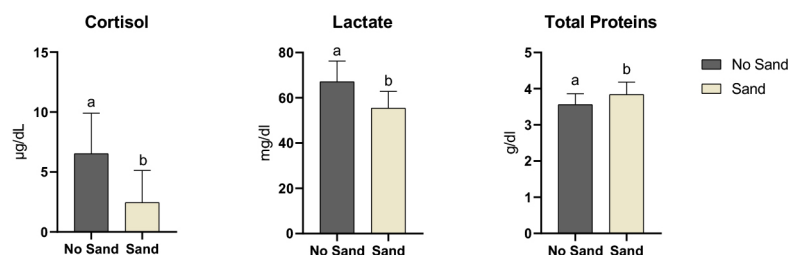


Figure 2. Cortisol, Lactate and Total Proteins of flathead grey mullet at two environmental enrichments (with/without sand). Different letters indicate significant difference.

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Results and Discussion

Environmental conditions significantly influenced fish growth performance (Figure 1) and serum biochemistry (Figure 2) of flathead grey mullet. The presence of sand appears to significantly modulate final body weight and specific growth rate. Feed conversion rate and feed intake were not influenced by environmental conditions. Cortisol and lactate levels were lower in EE fish, indicating reduced stress, while total protein levels were higher, suggesting improved nutritional and physiological status (Goikoetxea et al., 2021). These patterns suggest enhanced energy allocation and reduced chronic stress under EE conditions. Consistent with the natural presence of sand in the diet of wild individuals (Whitfield et al., 2012), these findings support the hypothesis that sand inclusion in recirculating aquaculture systems may improve digestive efficiency, likely by promoting proper gizzard functionality.

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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BIOFLOC-ENRICHED LIVE FEED IMPROVES GROWTH OF PIKEPERCH (*Sander lucioperca*) LARVAE

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Introduction

Pikeperch (*Sander lucioperca*) is a fast-growing freshwater predator with high-quality flesh and strong market demand (Policar et al., 2019). Its intensive larviculture nevertheless remains the main production bottleneck. Survival during the larval stages commonly falls below 20% under standard protocols, a loss attributed largely to inadequate rearing protocols and poor early nutrition (Yanes-Roca et al., 2020b). These problems are compounded by the biology of percid larvae, which hatch at a small size with a limited mouth gape that constrains the ingestion of live prey such as *Artemia* (Kestemont et al., 1996). The type and duration of the first live feed therefore strongly influence survival, growth and digestive-system development (Imentai et al., 2020, 2022), and enriching rotifers and *Artemia* has been shown to improve early growth and survival in pikeperch (Yanes-Roca et al., 2020a). Biofloc technology offers a complementary and sustainable route to improving larval feeding. It generates microbial aggregates that are rich in protein and fatty acids and contain bioactive, energy-rich compounds such as polyhydroxybutyrate, giving them a high nutritional value for cultured species (De Schryver et al., 2008; Khanjani et al., 2022). We therefore evaluated biofloc-enriched rotifers and *Artemia* against a standard live-feed control, hypothesising that it improves feed intake, growth and survival and fosters a more diverse and stable larval gut microbiota.

Materials and Methods

Pikeperch larvae were reared in a recirculating aquaculture system in four replicate tanks per treatment (525 larvae per 3.5 L tank; 150 larvae L⁻¹). Two treatments were compared. A control-fed standard live feed (rotifers and *Artemia*) and a Biofloc-enriched treatment fed the same live feed enriched in a separate system. The RAS system has temperature (16.6 ± 0.2 °C), dissolved oxygen (9.2 ± 0.1 mg L⁻¹) and salinity (5.5 ± 0.4 ppt). Larvae were sampled for total length (TL), eye diameter (ED), gut-fill score (0–4, expressed as % of maximum) and swim-bladder inflation. Fatty acid composition, oxidative-stress biomarkers and gut-microbiota (16S rRNA) analyses are ongoing. Data are presented as mean ± SD. Significant difference is presented as $p < 0.05$.

Results and Discussion

At 20 DPH, the two treatments differed markedly in larval size. Mean total length was greater in the biofloc-enriched group (11.28 ± 0.56 mm) than in the control (10.79 ± 0.40 mm; $p = 0.003$). Eye diameter followed the same trend, being larger in the biofloc-enriched larvae (0.82 ± 0.06 mm) than in controls (0.78 ± 0.05 mm; $p = 0.010$). This difference was not evident during early development however the treatments remained comparable in size until approximately 10 DPH, then diverged around 14 DPH, and the gap increased progressively thereafter until termination. In contrast, the remaining endpoints did not differ between treatments. Gut fill remained high in both groups, with no appreciable difference between the biofloc-enriched (91.3 ± 14.7%) and control larvae (88.8 ± 19.0%), indicating active feeding in both treatments. Swim-bladder inflation was equivalent, reaching 80% in each group. Survival was higher in the biofloc-enriched treatment (43.6 ± 13.1%) than in the control (29.9 ± 4.4%).

Biofloc concentrates microbial protein, lipids and bioactive compounds that enrich the nutritional profile of live prey, and in this trial those nutrients reached the larvae one trophic step up through gut-loaded rotifers and *Artemia* rather than by direct grazing (De Schryver et al., 2008; Khanjani et al., 2022). The larval performance improved by 20 DPH without any compromise to feed intake or swim-bladder inflation. Total length and eye diameter were both significantly higher under enrichment. The control TL of 10.79 mm matches the value reported for untreated pikeperch at 21 DPH by Yanes-Roca et al. (2020b). Gut fill exceeded 88% in both groups. Swim-bladder inflation reached 80% in each treatment, indicating that enrichment did not interfere with the surface air-gulping window that governs inflation success in pikeperch (Fazekas et al., 2021). The growth advantage was absent at first feeding and emerged only from around 14 DPH, in line with a cumulative effect of first-feed quality on pikeperch performance and digestive development (Imentai et al., 2020). Comparable benefits of biofloc as a live-feed supplement have been documented in other freshwater species like in *Betta splendens*, partial replacement of *Artemia* with ex-situ biofloc maintained growth and survival relative to *Artemia* alone (Stringhetta et al., 2024). These preliminary results identify biofloc-enriched live feed as a practical and sustainable route to improving early growth in pikeperch and reducing reliance on conventional enrichment products.

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FISH SIZING AND WEIGHT ESTIMATION USING STEREO CAMERAS ABOVE WATER FOR *Dicentrarchus labrax* AND *Oncorhynchus mykiss*

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Introduction

Aquaculture increasingly relies on precision monitoring to optimize feeding, size grading, and harvesting. Traditional physical sampling is invasive, labor-intensive, and induces acute stress, negatively impacting fish welfare and growth. While computer vision is transforming the sector (Yang et al., 2021), submerged systems are constrained in shallow or turbid land-based units by biofouling, light attenuation, backscatter, and maintenance demands. Above-water imaging avoids camera submersion but introduces a more complex optical problem because air-water refraction, ripples, foam, fluctuating water levels, and free-swimming behavior distort apparent fish geometry. This study presents an integrated above-water stereo vision methodology for estimating total length, biomass, and weight dispersion in European seabass (*Dicentrarchus labrax*) and rainbow trout (*Oncorhynchus mykiss*). **The framework advances stock-level monitoring by combining optimized stereo calibration, refraction-aware three-dimensional reconstruction, deep-learning contour extraction, curved-body morphometry, biological consistency filtering, and allometric weight conversion.**

Materials and Methods

The methodology is organized into a **development pipeline** and an **operational pipeline**. During development, stereo calibration was optimized using a custom cost function that minimized distance, boundary, and planarity errors from checkerboard images. The coordinate system was realigned so the XY-plane remained parallel to the water surface and the Z-axis represented water depth, reducing systematic reconstruction bias and enabling efficient two-dimensional refraction correction based on Snell's law instead of three-dimensional iterative ray tracing. YOLOv11x-seg models were trained for multi-fish and water-level instance segmentation using vertically stacked stereo pairs and iteratively refined annotations to preserve aspect ratios and improve contour recovery under occlusion and variable optical conditions. A Voronoi-based centerline algorithm measured naturally curved fish, overcoming straight-body assumptions common in earlier pipelines. Species-specific allometric models converted image-derived total length and body width into estimated weight.

During operation, synchronized stereo image pairs were acquired and processed with contrast-limited adaptive histogram equalization to reduce glare and improve visibility. The water-level model supplied dynamic depth parameters for refraction correction. Fish contours were encoded as translation-invariant polar descriptors anchored at the body center and matched between stereo views using K-Nearest Neighbors. After triangulation and optical correction, measurements were rejected when geometric or biological plausibility was violated, including unrealistic width-to-length ratios, excessive inclination, surface distortion, spatial inconsistency, partial occlusion, or merged silhouettes. Accepted detections were aggregated into population-level length and weight distributions rather than forcing estimates from unreliable frames.

Discussion and Conclusions

The system achieved high accuracy under realistic aquaculture conditions. Median total-length mean absolute percentage error reached 1.2% for rainbow trout and 2.3% for European seabass, while median-weight errors reached 4.9% and 6.9%, respectively. These results are comparable to leading underwater stereo systems (Tonachella et al., 2022) while avoiding submersion-related maintenance and visibility constraints. Optimized calibration was central to this performance, reducing measurement error from a maximum of 25% under standard OpenCV calibration to below 4.2% in all tanks. YOLOv11x-seg also surpassed the Mask R-CNN benchmark, reaching 98.2% mAP50 and improving contour recovery under partial overlap.

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In European seabass trials, the method remained effective despite turbidity up to 200 NTU, surface foam, and ripples, conditions representative of estuarine earthen ponds and shallow marine units where underwater imaging may be impractical. Automated water-level detection and dynamic refraction correction also overcame limitations of above-water systems that require fixed camera-to-surface distances. **The main contribution is a scalable, welfare-oriented framework that transforms above-water stereo images into reliable length and weight distributions for production management. By integrating 3D imaging, optical correction, robust segmentation, curved-body measurement, and morphological filtering, the method provides daily biometric data for feeding optimization, grading, growth modeling and harvest planning, which are key processes in Aquaculture 4.0 implementation.**

Acknowledgment

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UNCOVERING THE EPIGENETIC LANDSCAPE OF LARVAL ONTOGENY IN *Sparus aurata* - PROSPECTS FOR SHAPING DEVELOPMENT

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Introduction

Considerable efforts have been devoted to developing protocols aimed at driving farmed fish towards desired phenotypes. However, success has remained limited, likely because phenotypic programming is largely mediated through epigenetic mechanisms, while current knowledge on the epigenetic regulators (EpiRegs) that ultimately transfer environmental information to the genome remains scarce. Thus, there is a need to better understand the bases of this phenotypic plasticity to advance toward phenotypes that fit future aquaculture conditions. The main objectives of the present study were, first, to develop and validate a qPCR array for EpiRegs in gilthead seabream (*Sparus aurata*) and, second, to apply this tool to characterize, for the first time, the ontogenetic changes in EpiRegs during larval development of this species.

Materials and Methods

A qPCR array was specifically developed for *Sparus aurata* to profile epigenetic regulators. The design included 73 target genes identified as EpiRegs, followed by technical validation. The array was assembled in 384-well microplates and encompassed genes involved in key regulatory processes, including enzymes, enzyme regulators, transcription factors, transcription factor regulators, and methyl-binding proteins. Gene expression dynamics were assessed across four ontogenetic stages: before hatching, hatching, 6 days post-hatching (dph), and 20 dph. Besides, to assess gilthead seabream EpiRegs susceptibility to manipulation during early development, an additional experiment was conducted in which larvae were fed microdiets supplemented with an epigenetic modulator, and sampled at the same developmental stages.

Results

Initial results revealed that EpiRegs displayed ontogenetic expression patterns throughout larval development in gilthead seabream. Hierarchical clustering analysis identified several groups of genes sharing similar temporal dynamics across developmental stages (Fig. 1). While some EpiRegs showed high expression levels during hatching followed by a marked downregulation at later stages, others exhibited the opposite trend, with progressive activation during post-hatch development. Several key EpiRegs, including those involved in DNA methylation (e.g., *dnmt3ba*, *dnmt3l*, and *uhrf1*), chromatin remodeling and transcriptional repression (e.g., *mbd2*), growth regulation (e.g., *myca*), and DNA damage response (e.g., *gadd45b*), exhibited ontogenetic expression patterns during larval development. Furthermore, dietary supplementation with an epigenetic modulator altered the expression dynamics of several EpiRegs, modifying their natural ontogenetic profiles, indicating that gilthead seabream epigenome may be susceptible to nutritional modulation during critical developmental windows.

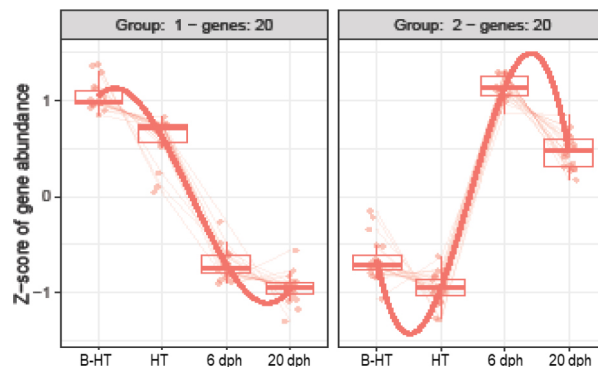


Figure 1. Hierarchical clustering analysis of EpiRegs expression profiles across larval developmental stages (B-HT: before hatching; HT: hatching; 6 and 20 days post-hatching) in gilthead seabream (*Sparus aurata*).

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Discussion

These results support the existence of a coordinated epigenetic regulatory program during early development in gilthead seabream. The ontogenetic variations observed in several EpiRegs highlights the dynamic nature of epigenetic regulation during critical developmental windows. Moreover, the modulation of these expression patterns by a dietary compound suggests that this regulatory program is susceptible to external nutritional cues in seabream. Ongoing analyses are currently addressing the relationship between EpiRegs expression patterns and tissue-specific changes in DNA methylation during development.

Together, these findings provide new insight into the molecular basis of developmental programming in fish and identify EpiRegs as promising targets for future nutriepigenomic strategies for precision nutrition in aquaculture.

Acknowledgment

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AN APPROACH TO STRUCTURE RISKS IN THE USE OF AUTOMATED SEA LICE ESTIMATIONS, USING SYSTEM-THEORETHIC PROCESS ANALYSIS

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Background

Automated sea lice monitoring in Norway are being increasingly utilized by fish farmers along the coast. The Norwegian Food Safety Authority (NFSA) have signaled the introduction of demanded use of the technology, which is also in line with Parliamentary developments. As a relatively new and rapidly developing technology with direct implications on regulation and compliance, there is a need to understand risk factors inherent in the technologies and connected to the use of it. This need is increased as the technology relies on artificial intelligence (AI), and there is currently a lack of standardization and comparability. System-Theoretic Process Analysis, “STPA” (Leveson & Thomas, 2018), is utilized as automated sea lice estimation is a socio-technical issue, in a control system including fish farmers and license holders, technology providers and regulators.

Method

The research group developed a methodology and conducted an analysis of the socio-technical control system, using STPA, building on the position paper “Sea Lice Monitoring of Tomorrow” (Usman et al., 2025). Through investigations into regulations, common practices, stakeholder discussions, and a regulatory sandbox hosted by NSFA, the analysis was developed to visualize and contextualize unsafe control actions (UCA).

Results and Conclusions

The STPA shows that data quality is a shared responsibility. The technology provider controls model design, uncertainty estimation, logic, updates, documentation, and support. The fish farmer controls daily operations, including placement, inspection, and maintenance, whereas the license holder controls procurement, procedures, and training. The regulator defines reporting expectations and compliance interpretation, whereas third-party assurance providers may provide verification, certification, or other assurance-related services. No single actor controls all factors that determine the reliability of the AI-generated sea lice estimation.

Operational conditions strongly affect data quality and require defined responses to poor-quality data and occasional manual verification. Effective use and fair regulations demand auditability, clear responsibilities and robust governance; without which the AI estimations risk misrepresenting the real sea lice situation in the pen and farm, and weak compliance, also despite accurate models.

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SEASONAL WARMING AND RANDOM STRESSORS ALTER BIOLOGICAL AGEING- AND THERMAL-RESPONSIVE GENE EXPRESSION IN GILTHEAD SEA BREAM (*Sparus aurata*)

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Introduction

Advancing sustainable aquaculture requires robust and quantifiable tools to assess fish welfare and physiological resilience under farming conditions (Noble et al., 2026). In this context, biomarkers capable of capturing the cumulative effects of environmental and husbandry-related stressors are becoming increasingly relevant. Among these, metrics of biological ageing have emerged as a promising framework for evaluating organismal condition beyond chronological age, whereas temperature-responsive molecular markers provide key insight into the adaptive capacity of farmed fish under warming scenarios. Using an epigenomic-transcriptomic integrative approach, recent studies in gilthead sea bream identified and validated 10 biological ageing genes (BAGs: *atp1a2*, *bmp1*, *calcr1*, *col5a1*, *psmd1*, *ramp1*, *sirt1*, *smad1*, *spred2*, *thrb*) and 36 temperature response genes (TRGs: *adam8*, *arrdc3*, *egr1*, *hmgcr*, *igf1*, *igf1r*, *jund*, *mapk1*, *mfn2*, *mmp14*, *nolc1*, *pcna*, *seidb1*, *sirt1*, *slc27a4*, *smarcd1*, *snx17*, *spred2*, *stk11*, *tymc*, *ucp3*, *usp47*, *usp7*, *cat*, *cox1*, *cpt1a*, *cs*, *ghr1*, *ghr2*, *gpx1*, *grp170*, *grp94*, *hif-1a*, *igf2*, *prdx3*, *sod2*) in white skeletal muscle (WSM) (Belenguer et al., 2024; Naya-Català et al., 2025). Building on these findings, the present study investigated the combined effects of water temperature and random stressors on the expression of these marker panels, and examined their relationship with growth performance and circulating biochemical indicators.

Materials and methods

Gilthead sea bream juveniles (~20 g) from the same production batch were acclimated in 3000 L tanks for 21 days. On 26/05/2025 (t₀; 21.35 °C), fish were redistributed into duplicate tanks assigned to a control group (CTRL), maintained under standard farming conditions, or to a stress group (STR), exposed to random stressors applied in variable order and timing to avoid habituation. During the first phase (t₀–t₁; 09/07/2025; 27.6 °C; 80–100 g), STR fish were subjected to a moderate stressor load (16 min/day), including chasing (3 × 1 min, with 3 min between series, after reducing water level), darkness (20–30 min), noise (3 × 10 impacts, with 10 min between series), and confinement (~75% volume reduction for 30–45 min). As no clear growth differences were detected, stress intensity was increased during t₁–t₂ (27/08/2025; 28.56 °C; ~150 g) to 33 min/day. In this phase, stressors were applied twice daily: chasing was performed with two nets against the swimming direction of the fish (3 × 2 min, with 4 min between series), darkness lasted 30–45 min, noise increased to 3 × 20 impacts with 2 min between series, and confinement was applied for 45–60 min. No further stressors were imposed from t₂ to the end of the experiment (t₃; 03/10/2025; 24.57 °C). At each sampling point (t₀–t₃), 12 fish per group were anesthetized and sacrificed by cervical section. Caudal blood was collected to determine total antioxidant capacity, hemoglobin, glucose, and cortisol. Dorsal WSM samples (150–200 mg) were preserved in RNeasy lysis buffer for RNA extraction and automated quantitative PCR analysis. Temporal and between-group differences were assessed using one-way ANOVA and t-tests (p < 0.05). Multivariate analyses included PERMANOVA (FDR < 0.05), PLS-DA (pR2X < 0.05; pQ2 < 0.05), and hierarchical clustering based on individual fold-change profiles, with markers showing VIP ≥ 1 and p < 0.05 selected as discriminants of group separation.

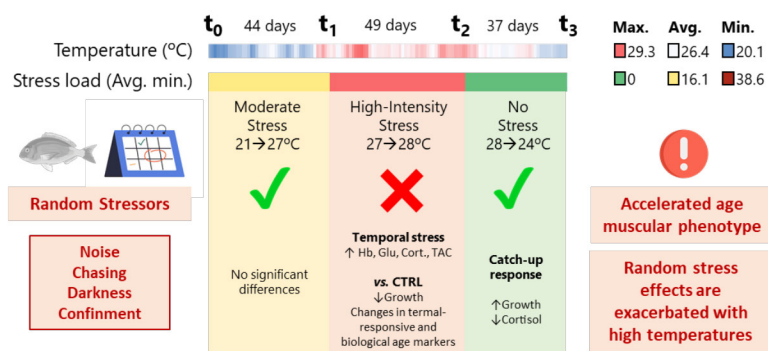


Fig. 1. Graphical abstract of the main results in this study.

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Results and Discussion

Over the course of the trial, fish increased in body weight from ~20 to ~280 g. The clearest effect of the combined stress–temperature challenge was observed at t2, when STR fish showed an 8% reduction in growth, coinciding with intensified random stress exposure and the highest seasonal water temperatures (Figure 1). This was accompanied by a 15% decrease in feed intake and an 18% reduction in hepatosomatic index, indicating a marked energetic and physiological adjustment under cumulative challenge. Blood parameters also showed a strong time-dependent pattern, with the greatest changes at t2: haemoglobin increased from 4.5 to ~10 g/dL, glucose from ~50 to ~150 mg/dL, and cortisol from ~50 to ~150 mg/dL, whereas total antioxidant capacity decreased from ~1200 to ~800 μ M Trolox. However, these variables did not discriminate between CTRL and STR fish at t1 or t2, suggesting that seasonal warming was the main driver of the systemic response, potentially masking the specific contribution of the imposed stressors. In contrast, WSM transcriptional profiling was more sensitive in detecting treatment-related effects. The number of differentially expressed genes between CTRL and STR was highest at t2, with 9 DEGs, compared with 3 and 4 at t1 and t3, respectively. Similarly, PERMANOVA of fold-change signatures separated t2 from the remaining time points, and PLS-DA confirmed this pattern, identifying 19 genes ($VIP \geq 1$ and/or $p < 0.05$) as the main contributors to group segregation. In the STR group, 4 BAGs (*atp1a2*, *bmp1*, *col5a1*, and *spred2*) and 10 TRGs (*cat*, *grp170*, *hif1a*, *igf1*, *igf1r*, *igf2*, *mmp14*, *nolc1*, *snx17*, and *spred2*) were downregulated, consistent with molecular signatures previously linked to accelerated biological ageing and thermal stress responses. These findings indicate that WSM transcriptomic markers can detect subtle but biologically relevant effects of the interaction between chronic random stress and elevated temperature, even when classical blood indicators fail to do so. After stressor exposure ceased, STR fish showed a 6.8% increase in growth at t3, together with a 50% reduction in circulating cortisol relative to CTRL, suggesting compensatory growth and partial physiological recovery. Overall, these results confirm the relationship between TRGs, BAGs, random stress, and temperature fluctuations, and support the idea that the combination of sustained stress and high temperature may act as an indicator of accelerated ageing and welfare maladaptation, particularly under the environmental pressure of the warm season.

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THERMAL HISTORY DIFFERENTIALLY SHAPES THE GUT MICROBIOME RESPONSE OF GILTHEAD SEABREAM (*Sparus aurata*) JUVENILES UNDER WARMING CONDITIONS

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Introduction

Aquatic organisms closely interact with their environment and associated microorganisms, driving growing interest in microbiota–host–environment interactions. Farmed fish are particularly sensitive to rearing conditions, as ectotherms whose growth, welfare and disease resistance are strongly affected by water temperature. This is especially relevant in Mediterranean aquaculture, where the semi-enclosed Mediterranean Sea is highly vulnerable to global warming and gilthead seabream (*Sparus aurata*) reared in sea cages are directly exposed to seasonal fluctuations and extreme warming events. Recent evidence showed that extreme summer heat episodes can induce marked intestinal microbiota shifts in this species (Domingo-Bretón et al., 2025). However, responses to thermal stress may depend not only on the maximum temperature reached, but also on the previous thermal trajectory experienced by the fish. Thus, this study aimed to evaluate whether differential pre-acclimation can modulate the intestinal microbiome response of gilthead seabream exposed to a subsequent acute temperature increase.

Materials and methods

Gilthead seabream juveniles were acclimated from April to May 2025 in an open-flow system under natural photoperiod and temperature at the IATS facilities (40°5'N, 0°10'E). Then, 220 fish were randomly distributed into ten 90 L tanks connected to two RAS systems (22 fish per tank). After 10 days at 20 °C, baseline intestinal microbiome sampling was performed before temperature rise (t₀). Tanks were assigned to two thermal regimes, each with five replicate tanks: Warming Programmed (WPr) and Warming Abrupt (WAb) (Figure 1A).

In WPr, temperature was progressively increased from 20 °C to 24 °C, whereas WAb remained at 20 °C. After 12 days, intermediate sampling was performed (t₁). Temperature was then increased to 30 °C in both treatments and maintained for three weeks until final sampling (t₂). Growth parameters were recorded at each sampling point, and anterior intestinal samples were collected for DNA extraction from 10 fish per group/sampling point. Full-length 16S ribosomal RNA gene were sequenced in a PromethION P2 of Oxford Nanopore platform. Reads were basecalled with SUP model of Dorado v1.4, and taxonomic assignment was performed with minimap2 against SILVA v138.2.

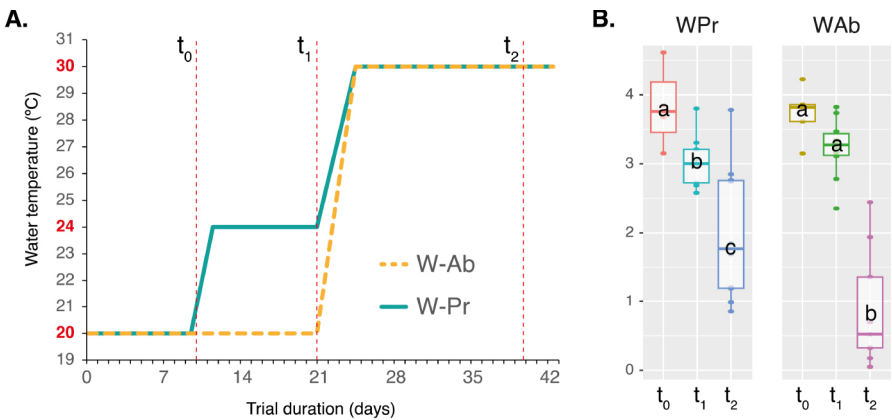


Fig. 1. (A) Representation of both thermal trajectories performed (B) Alpha diversity (Shannon index) between sampling points (t₀-t₂) for both thermal regimes.

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Results and discussion

Specific growth rate (SGR) did not differ between groups during the initial period, indicating comparable starting conditions before temperature differentiation. During the second period, WPr showed higher SGR than WAb, consistent with its rearing in warmer water during programmed acclimatation. Notably, during the final period, when both groups were maintained at 30 °C, SGR remained higher in WPr, suggesting an effect of the previous thermal trajectory and a reduced growth response after abrupt warming in WAb.

Regarding the gut microbiome, alpha diversity showed a temperature-associated decrease (Figure 1B). In WPr, significant differences were observed among all sampling points, indicating gradual restructuring during programmed warming. In WAb, no differences were detected between t0 and t1, consistent with both points being maintained at 20 °C, but diversity declined significantly after the abrupt increase to 30 °C. At the final sampling point, diversity was significantly lower in WAb than in WPr, suggesting that programmed warming mitigated the loss of microbial diversity after exposure to 30 °C. At the phylum level, Pseudomonadota dominated the anterior intestinal microbiome, followed by Bacillota and Actinomycetota. In WPr, phylum-level composition remained comparatively stable, with a transient increase in Actinomycetota at t1. In WAb, no significant differences were detected between t0 and t1, whereas t2 showed a strong taxonomic shift, with Pseudomonadota increasing to 95.2% and displacing Bacillota, Actinomycetota and Bacteroidota phyla. Bray-Curtis-based PERMANOVA revealed significant shifts in microbial community structure in both thermal regimes. In WPr, all pairwise comparisons were significant, with moderate and similar effect sizes, supporting gradual microbiome differentiation. In WAb, the strongest changes occurred after the abrupt increase to 30 °C, with higher effect sizes in the t1 to t2 transition than in WPr. At genus level, the final reduction in diversity was associated with Pseudomonadota-affiliated taxa in both systems, but in WAb this shift was mainly driven by *Enterovibrio*, which dominated the final microbiome and represented more than 80% of the community. This finding agrees with previous evidence in greater amberjack (*Seriola dumerili*), where *Enterovibrio* was associated with high-temperature microbiome shifts under marine heatwave conditions (Sánchez-Cueto et al., 2023). Overall, these results suggest that acute warming reduces intestinal microbial diversity in gilthead seabream juveniles, while the magnitude and structure of the microbiome response depend on previous thermal background.

Funding

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EFFECTS OF IMAGE QUALITY AND INTER-OBSERVER VARIABILITY ON IMAGE-BASED SALMON LICE DETECTION

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Introduction

Accurate estimation of salmon lice (*Lepeophtheirus salmonis*) levels is essential for fish welfare, farm management, and regulatory compliance in aquaculture. While traditional manual counting methods are still widely used, they rely on small sample sizes and may not always provide representative estimates (Jeong et al., 2021). Recent years have seen the development of camera-based solutions that identify salmon lice using dedicated machine learning models. Such image-based monitoring systems offer a non-invasive alternative, enabling larger sample sizes and more frequent observations (Rabe et al., 2024). However, image-based identification remains challenging due to the small size and varying life stages of salmon lice and the variation in image quality that occurs under commercial conditions. Moreover, detector training still depends on human annotation, which can vary between individual analysts. Such inter-observer variability may affect the performance of automated detection systems trained on these data (Quinn et al., 2023). This study evaluates the consistency of lice counting among trained analysts to better understand how this variability may influence image-based monitoring.

Materials and methods

Image sequences of Atlantic salmon (*Salmo salar*) were sampled from a representative selection of farming conditions using a commercial underwater camera system (Stingray Marine Solutions AS). A total of 2,160 sequences were initially assessed for image quality by two independent analysts based on visibility, lighting, and sharpness. Only sequences with agreement classified as Good or Fair quality were included for further analysis. Salmon lice were then counted and classified independently by three experienced analysts using standardized guidelines, distinguishing between adult female and mobile lice (adult males and pre-adult stages I and II of both sexes). During lice counting, analyst uncertainty was also recorded. Inter-observer agreement was evaluated using Cohen's kappa for quality assessment and the intraclass correlation coefficient (ICC) and exact agreement for lice counts.

Results

Agreement between analysts on image quality classification was moderate, with 63% of sequences consistently classified. Salmon lice count showed high inter-observer reliability across both lice stages and image quality levels (ICC > 0.93). Exact agreement between analysts was high overall, but lower for mobile lice and Fair-quality sequences compared to adult females and Good-quality sequences. Uncertainty in lice detection and stage classification was significantly higher in Fair-quality sequences.

Discussion

The results demonstrate that trained analysts can produce highly consistent salmon lice counts from image sequences, with excellent inter-rater reliability across lice stage groups and image quality levels. Although exact agreement was lower, particularly for mobile lice and Fair-quality images, most differences between analysts were small, indicating that variability was largely random rather than systematic.

Image sequence quality was a key driver of variability. Reduced quality increased uncertainty in both salmon lice detection and stage classification, highlighting its impact on reliable interpretation. Despite this, overall counting patterns remained stable, suggesting that experienced analysts can maintain consistency even under less optimal conditions.

These findings are important for automated detection and monitoring under commercial farming operations. Consistent human annotations provide a strong foundation for machine learning, but lower image quality can reduce sensitivity, especially for the smaller mobile lice stages and under challenging visibility conditions. Overall, image-based monitoring can provide robust and scalable estimates based on large sample sizes when image quality and inter-observer variability are taken into consideration.

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COMPARATIVE GOVERNANCE PATHWAYS FOR SUSTAINABLE INLAND AQUACULTURE DEVELOPMENT: LESSONS FOR THE BALTIC STATES

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Introduction

The development of sustainable inland aquaculture has become increasingly important in the context of global food security, climate adaptation and the transition towards resilient blue and bioeconomy systems (European Commission, 2021; FAO, 2024; Puskarski & Śniadach). While the European Union promotes aquaculture as a strategic sector within the Farm-to-Fork Strategy (European Commission, 2020), inland aquaculture development in the Baltic States remains comparatively limited and structurally fragmented (Barazzetta, 2024; Riekstiņš, 2024).

At the same time, several European and non-European countries have developed governance approaches and production models that may provide relevant lessons for the Baltic region, particularly concerning small and medium-sized enterprises (SMEs), innovation, environmental sustainability and policy coordination.

This presentation introduces the comparative governance dimension of the interdisciplinary research project “*Potential of traditional and recirculation inland aquaculture for inclusive and intelligent fisheries development in the Baltic States (AQUA4DEV-BS)*”. The presentation focuses on the conceptual framework for analysing governance pathways and institutional arrangements influencing inland aquaculture development across selected countries and their potential applicability to the Baltic States.

Materials and methods

The study applies a comparative interdisciplinary research approach combining comparative governance analysis, legal and policy analysis and case-study methodology. The research examines inland aquaculture governance frameworks in selected European countries (Hungary, Poland, the Czech Republic and Denmark) and selected non-European reference cases (Namibia, Bangladesh and Brazil).

The comparison focuses on several analytical dimensions relevant to sustainable inland aquaculture development, including:

- regulatory and institutional governance structures;
- support mechanisms for SMEs;
- environmental sustainability and climate adaptation measures;
- integration of aquaculture into bioeconomy and rural development policies;
- innovation and technological modernization, including recirculating aquaculture systems (RAS);
- stakeholder participation and governance coordination.

The study combines analysis of legal and policy documents, strategic development plans, scientific literature and sectoral reports with expert-based qualitative insights generated within the AQUA4DEV-BS project framework.

The comparative analysis is further supported by elements of Actor-Network Theory, allowing examination of the interactions between public institutions, industry stakeholders, environmental requirements, technological systems and market conditions shaping inland aquaculture governance.

Expected Outcomes and Discussion

The research is expected to identify different governance pathways influencing inland aquaculture development and to highlight transferable practices relevant for the Baltic States. Preliminary analysis suggests that successful inland aquaculture development depends not only on production technologies but also on governance coordination, administrative flexibility, targeted support mechanisms and long-term policy stability.

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The study is expected to demonstrate that countries with more integrated governance approaches tend to provide stronger institutional support for aquaculture SMEs, facilitate the uptake of innovation, and improve sector resilience under conditions of environmental and economic change.

The comparative perspective may also reveal important differences between governance approaches in traditional pond aquaculture systems and technologically intensive RAS systems, particularly concerning environmental regulation, investment structures and stakeholder participation.

The findings will contribute to the development of the AQUA4DEV-BS decision-support tool and provide practical recommendations for strengthening sustainable inland aquaculture governance and resilience in the Baltic States.

Acknowledgment

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EVALUATION OF PILOT-SCALE LIQUID MICROALGAL CONCENTRATES FOR *Magallana gigas* LARVAL CULTURE

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Introduction

The availability of nutritionally adequate and reliable diets is crucial to the success of hatchery production of Pacific oyster (*Magallana gigas*) larvae. Mixed diets based on live microalgae, including *Tisochrysis lutea* and *Skeletonema costatum*, are widely used because of their complementary nutritional profiles and suitability for different stages of larval development [1,2]. However, their continuous production is costly, technically demanding, and highly variable in culture performance and vulnerable to contamination [2]. Therefore, microalgal concentrates may represent a useful tool to overcome these limitations, providing practical and standardised alternative diets. This study evaluated the potential of pilot-scale liquid concentrates of *T. lutea* and *S. costatum* as alternatives to conventionally cultured live microalgae for feeding Pacific oyster larvae.

Material and Methods

Pacific oyster larvae, 72 h post-fertilization, were randomly distributed in 20 L tanks at an initial density of 7 larvae ml⁻¹. Larvae were cultured for 18 days in closed systems with UV-sterilized filtered seawater and continuous aeration. Water was renewed every two days and temperature was maintained at 20±1°C. Four dietary treatments were evaluated in triplicate: 1) live microalgae diet containing *T. lutea* and *S. costatum* (control); 2) Experimental Diet 1 (ED1), liquid concentrate of *T. lutea* and *S. costatum* at 4% dry weight (DW); 3) Experimental Diet 2 (ED2), liquid concentrate of the same microalgal species at 4% DW, subjected to thermal treatment; 4) unfed treatment (negative control). The daily feeding ration and the relative contribution of each microalgal species were progressively adjusted according to larval age (table 1). ED1 and ED2 were formulated by Necton – Portuguese Company of Marine Cultures S.A.. Larvae were sampled three times per week to assess survival and growth, based on shell length. Statistical analyses were performed using R version 4.5.3. One-way ANOVA, followed by Tukey post-hoc test ($p < 0.001$) was used to evaluate differences among dietary treatment.

Results and Discussion

By the end of the experiment, larvae fed with the control diet exhibited significantly better performance than those fed the experimental diets, with significantly higher survival rates, greater shell growth (Figure 1) and the highest specific growth rate (SGR; 7.3 ± 0.23 % day⁻¹). In contrast, ED1, ED2 and the unfed control exhibited markedly lower SGR values (0.33 ± 0.10 , 0.52 ± 0.26 and 0.76 ± 0.11 % day⁻¹, respectively). No significant differences in survival or shell length were detected among ED1, ED2 and the unfed control at 21 days post-fertilization. Higher product doses may be required, as observed for commercial microalgal diets in other species [3]. ED2 showed a transient advantage over non-thermally treated ED1 up to day 15, possibly due to a reduced bacterial load and/or increased nutrient bioavailability. Future trials will optimize product dosage to improve larval performance.

Acknowledgment

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Table 1. Daily feeding ration and relative proportions of *Tisochrysis lutea* and *Skeletonema costatum* supplied to the fed treatments (Control, Experimental Diet 1 and Experimental Diet 2) throughout the experimental period. DW = dry weight.

Experimental days	<i>T. lutea</i> (mg DW ml ⁻¹)	<i>S. costatum</i> (mg DW ml ⁻¹)
1-5	6.10×10^{-4}	0
6-8	9.15×10^{-4}	5.22×10^{-4}
9-15	9.15×10^{-4}	1.57×10^{-3}
≥ 16	7.63×10^{-4}	3.92×10^{-3}

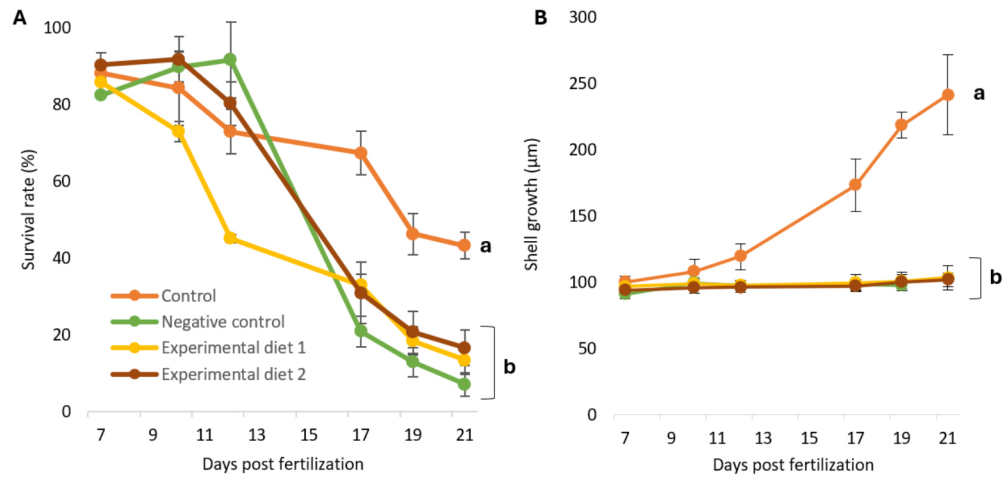


Figure 1. Survival rate (A) and shell growth (length) (B) of *Magallana gigas* larvae fed with four dietary treatments throughout the experimental period. Data were expressed as mean $\pm$ SD (n=3). Different lowercase letters indicate significant differences among treatment at 21 days post-fertilization (One-way ANOVA, Tukey test $p < 0.001$).

ADDING TO THE UNDERSTANDING OF DIRECT GREENHOUSE GAS EMISSIONS FROM AQUACULTURE FACILITIES

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Introduction

The global warming potential (GWP) of the net carbon and greenhouse gas (GHG) balance associated with food production is a high priority for many consumers, who are increasingly adopting a more vegetarian diet, reducing demand for meat and seafood options. “Seafood” (from fresh or marine water) typically has a lower GWP than terrestrial animal protein options, but there is high variability. The net GHG balance of seafood production forms a further aspect of environmental impact, but has so far not been considered consistently, particularly direct emissions from aquaculture ponds. The main GHGs of interest from food production are methane and nitrous oxide which both have significant impacts on global warming. Carbon dioxide is mostly biogenic in farming systems which is considered to be neutral because it is sequestered in feed ingredients and then released again by the cultured animal through respiration. Usually of more concern are methane and nitrous oxide that have 34 and 298 times the radiative forcing effect of CO₂ respectively. Early papers concerning GHG emissions from aquaculture relied on assumptions with direct measurements reported only in recent years and there is still a significant gap in our knowledge of the greenhouse gas balance of different aquaculture systems, because of the huge variability of species and systems. This paper seeks to add to the discourse on the generation of GHGs from shrimp aquaculture facilities. In shrimp ponds, methanogenesis may not be the most energetically favourable pathway (Rowe et al., 2022). In saline environments, anaerobic decomposition preferentially uses sulphate as an intermediary leading to H₂S production, but reverts to methanogenesis once sulphate is depleted or, if sediment organic loading is high and there is no competition for substrate, methanogenesis can co-exist with sulphate reduction (Sela-Adler et al., 2017). Therefore the relationship between salinity and organic loading in shrimp ponds was hypothesized to be a key determinant of methane emissions.

Methods

We took 291 separate flux measurements and corresponding data on salinity and organic matter content from shrimp farms in Ecuador and Honduras, spanning a range of salinities and organic loading. Chambers were deployed at various points within ponds to achieve representative sampling. Gas samples were collected using a floating chamber with a plastic tube attached, from which gas samples could be extracted. Samples were collected using a syringe and injected into pre-evacuated exetainers for later analysis by gas chromatography. Gas samples were analysed using a Hewlett Packard 5890 series II gas chromatograph (GC) fitted with Flame Ionisation and Electron Capture Detectors, both at 350°C. Gases were separated on a porous polymer column (HayeSep® Q, Valco Instruments, Houston, USA). Samples were run against standards of CO₂, CH₄ and N₂O of known concentrations. The rate of GHG production was calculated on the basis of the linear increase in amounts of the respective gases in the volume of the gas collection chamber.

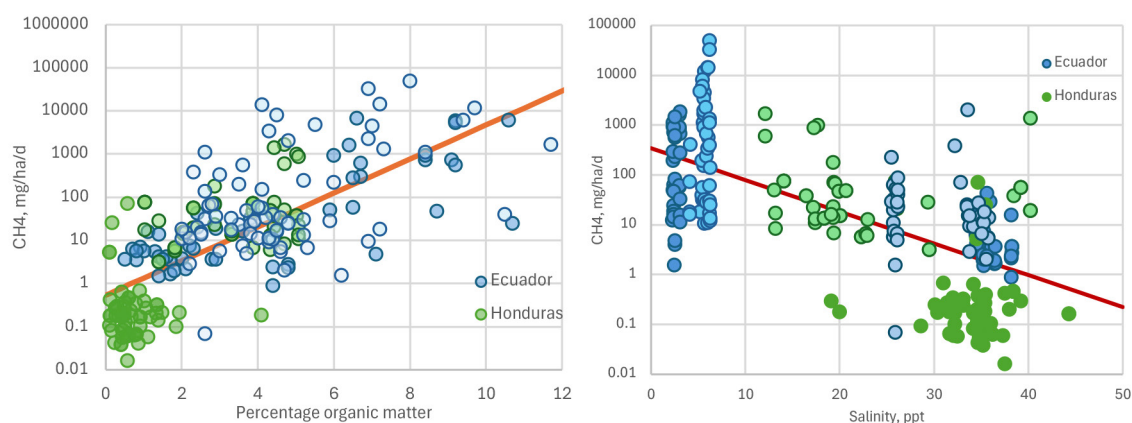


figure 1. Methane emissions from tropical shrimp ponds related to a) organic matter loading and b) salinity.

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Results and discussion

Clear relationships were observed between both salinity and organic matter (Fig 1) with methane emissions rising steadily with organic loading. However, higher salinity inhibited methane emissions considerably with highest salinities demonstrating little methane generation, even at high organic loading. It was estimated that for every 10ppt rise in salinity, there was an approximately 60% lowering of methane emissions. The analysis confirmed that salinity is a major determinant of methane generation, although with high uncertainty. Seasonality and fluctuating affects of rainfall may have an effect on salinity and organic loading, or other water quality parameters, which are an important area for further research. Very few nitrous oxide emissions were observed at any rate of organic loading or salinity.

Acknowledgment

We would like to thank Laurence Massaut and Louis Cattini for their diligence and hard work collecting gas samples from Ecuador and Honduras respectively.

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LIFE CYCLE ASSESSMENT OF CHINESE CARP POLYCULTURE SYSTEMS FROM THREE PROVINCES

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Introduction

China produces is, by far, the largest producer and consumer of farmed fish in the world, including a large variety of freshwater and marine finfish, molluscs, crustaceans and plants, representing around 70% of global aquaculture production. Chinese carps (grass carp, bighead carp, crucian carp, common carp and silver carp) and tilapia are the most cultured finfish species, produced in varied polycultures, often integrated with terrestrial livestock. Although most carp are produced for domestic markets, their production relies on global supply chains with far-reaching impacts.

Production grew rapidly from the1980s and has underpinned the “Blue Revolution”. However, very little is published on Chinese aquaculture production and there has been almost no publication in international literature concerning the environmental efficiency or impacts of this production. We present a comprehensive Life Cycle Assessment of Chinese carp production covering the three largest producing provinces (Hubei, Jiangsu and Guangdong) with primary data collected from over 100 farms in each province, including feed use, production intensity, energy and fuel use.

Methods

Major aquaculture producing areas within the three largest carp producing provinces were targeted for data collection with a hyndred farms surveyed in each of Hubei, Jiangsu and Guangdong. A comprehensive survey was constructed, detailing critical inputs and outputs to conduct an LCA, including aquaculture production, feed use, electricity, fuel, fertiliser and chemical use. Data was also collected from aquaculture production facilities on sustainability perceptions, stock losses, labour and costs. Feed ingredient data was also collected from two large feed producers for a standard carp polyculture and monoculture feed. Data was cleaned and classified into different scales and production types before being modelled in Simapro software. Methods followed the EU PEFCR approach wherever possible using economic allocation for feed resources and for co-produciton at the aquaculture facility. Agifootprint 7.0 and Ecoinvent 3.12 databases were used for feed and energy inputs respectively.

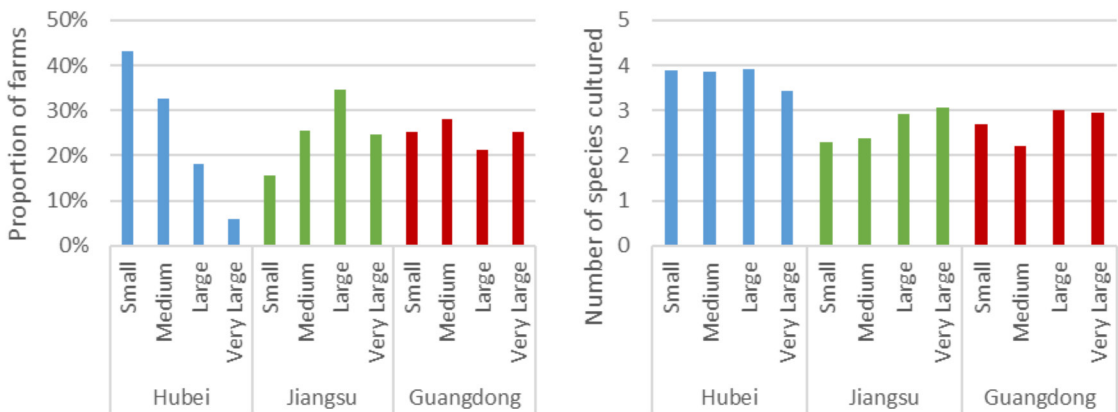


Figure 1. Distribution of different scale aquaculture farms between 3 Chinese provinces (a) and average number of species cultured (b)

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Results and discussion

Most farms were polycultures of several fish species, most typically with grass carp (*Ctenopharyngodon Idella*), silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Hypophthalmichthys nobilis*) as the most common, traditional combination. Crucian carp (*Carassius carassius*) was the next most cultured species, either in monoculture or as a polyculture, with far fewer farms culturing other species. However, in Guangdong, many farmers were culturing mud carp (*Cirrhinus molitorella*) which was used as live feed for mandarin fish (*Siniperca chuatsi*). There were some other important differences between provinces. Guangdong produced fish more intensively and farms were often larger scale compared especially to Hubei which had lower stocking rates, smaller farms and were generally more diverse (Figure 1). Feed is often by far the largest contributor to Life Cycle impacts of aquaculture production (Newton and Little 2018) and this was also the case for Chinese carp with over 90% of climate change, land and water use impacts. However, eutrophication at the farm production site was also a major issue, especially at the very intensive farms in Guangdong. Overall impacts from carp production were inline with other aquaculture species. However, much of this was related to high eFCRs (ave 2.27) and the actual impact of feed was low compared to some other aquaculture species. Therefore, impact could be reduced substantially by improving eFCR by several means. The Chinese government has been concerned with reducing the impact from aquaculture which is regarded as a major cause of eutrophication and evidence suggests that many aquaculture areas are being converted to other industries or even rewilded (Newton et al 2021), perhaps with a more conscious strategy of zonation to avoid conflicts between stakeholders.

Acknowledgment

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SMART ELECTRIC AERATION AS A PRECISION TECHNOLOGY SOLUTION FOR OPTIMISING WATER QUALITY, FISH HEALTH, WELFARE AND PERFORMANCE IN ATLANTIC SALMON *Salmo salar* AQUACULTURE

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The increasing adoption of precision, sensor-driven technologies in salmon aquaculture is driving improvements in operational efficiency, fish welfare and environmental sustainability. Aeration is a routine requirement in Atlantic salmon net-pen aquaculture used to maintain adequate dissolved oxygen during periods of compromised water quality, disease events and medicinal or mechanical treatments. Currently, aeration is typically provided by centralised diesel or electric compressors whose operating costs restrict their use to critical periods leaving fish to cope with potentially suboptimal oxygen conditions at other times.

This study evaluated a compact, portable, single-phase electric aeration unit with on-demand, sensor-triggered control as a precision alternative to standard centralised electric aeration in an 8-month commercial-scale trial at an Atlantic salmon net-pen site. Six pens were randomly assigned to one of two treatments ($n = 3$ per treatment): on-demand electric aeration triggered automatically by a low-oxygen or high-chlorophyll threshold, or centralised electric compressor aeration applied as per standard husbandry practice. Water quality was monitored continuously across pen volume and depth using dissolved oxygen, temperature, chlorophyll and salinity data loggers. Fish health and welfare were assessed via weekly health checks, including gill scoring for amoebic gill disease (AGD) and proliferative gill disease (PGD), sea lice counts and physical condition scoring. Production performance metrics, including growth, feed conversion ratio, feed consumption and mortality, were collected from farm records throughout the trial. Chronic stress was assessed by measuring scale cortisol at four time points using HPLC-MS/MS (April, July and November) with 15 salmon sampled per time point across all experimental pens.

A full life-cycle assessment (LCA) was conducted to establish and compare the environmental credentials of the two aeration systems, conforming to ISO 14044 methodology and considering EU Product Environmental Footprint Category Rules (PEFCR) where applicable. Greenhouse gas emissions were separated into Scope 1, 2 and 3 with full contribution analysis to identify efficiency improvement targets for each system. Standard PEF impact categories were assessed, including Global Warming Potential, Acidification Potential and Eutrophication Potential, alongside aquaculture-specific indicators such as Fish In Fish Out ratio and welfare metrics including mortality per tonne of fish produced.

Results will be presented comparing water quality profiles, fish health and welfare indicators, production performance and full life-cycle environmental impacts between on-demand precision electric aeration and standard centralised electric aeration across a full production cycle. This study provides the first rigorous commercial-scale evaluation of precision, sensor-triggered aeration as a smart aquaculture technology and assesses its potential to improve fish outcomes, operational efficiency and environmental sustainability within the broader transition to integrated, data-driven salmon production systems.

CONSUMER PERCEPTIONS OF SUSTAINABILITY INNOVATIONS IN EUROPEAN FRESHWATER AQUACULTURE

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Introduction and objectives

The SmartAqua4FuturE (SAFE) project is researching circular bioeconomy innovations to improve the sustainability of European freshwater aquaculture. Consumer perceptions and purchasing behaviours are an important driver for, or barrier to, the adoption of these innovations. An online survey was therefore designed to provide some assessment of consumer attitudes to fish consumption across Europe. This was followed by a series of focus groups to provide greater insights into consumer perceptions and priorities.

Approach and methods

Mentimeter.com was used for the online survey platform due to the modern graphical interface this provided for participants. Six language versions were made available (English, Spanish, Portuguese, Polish, Finnish and Swedish) based on the SAFE project partner countries. The questions covered participant demographics, fish consumption habits over the previous month, perceptions of aquaculture practices and attitudes towards different circular bioeconomy technologies and potential policy measures to improve sustainability. As no budget was allocated for this activity it was not possible to implement a representative sampling frame for such a large potential population. Recruitment was therefore self-selecting based on promotion through numerous social media channels, initially in July 2025 with a further smaller round in January 2026. The results of the online survey were then analysed and used to inform a series of focus groups held in the partner countries during May and June 2026. These involved different demographic groups, but all consumers of fish and not directly involved in the seafood sector.

Survey results

A total of 592 responses were received in the online survey with an average 93% completion rate excluding the two final open-answer questions. In terms of demographics, 29% of respondents were from Southern Europe, 26% from Western Europe, 20% from Nordic countries, 9% from Eastern Europe, 12% from unidentified European countries and 4% from elsewhere. All adult age ranges were represented with a broadly normal distribution around middle-age. 95% of respondents were at least occasional fish eaters of which 81% were regular fish eaters. The average consumer recorded 14 fish purchases over the previous month led by salmon, seabass or seabream (average almost three times per month) with freshwater fish purchased on average once per month. In terms of sustainability, the respondents scored aquaculture produced fish above all other animal proteins and pond fish above tank-reared fish which in turn scored above cage-reared fish. This scoring appeared to be influenced by sources of information available to the respondents. All aquaculture systems were perceived more favourably by respondents who obtained their information from colleagues or formal education and less favourably by those who relied on friends, mainstream, and particularly social media. Local production and production systems which appeared more “natural” were favoured with the recycling of byproducts into feed ingredients less so. Policy measures aimed at supporting greater sustainability that were likely to increase price to consumers were viewed much less positively by consumers who already considered seafood to be expensive or unaffordable than those who considered it to be affordable.

Discussion

The results suggest that aquaculture practices that appear closer to “natural” production are favoured by consumers, but tempered by their budget and hence willingness to pay a premium for this.

Acknowledgment

The SmartAqua4FuturE project has received funding from the European Union’s Horizon Europe programme under grant agreement no. 101084549. The University of Stirling’s contribution is funded by Innovate UK through the Horizon Europe Guarantee Fund.

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LIFE CYCLE ASSESSMENT OF BLUE MUSSEL (*Mytilus edulis*) AS INGREDIENTS FOR FARMED ATLANTIC SALMON (*Salmo salar*)

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Introduction

European aquaculture is especially vulnerable to supply chain shocks related to feed provision. Over 90% of Norwegian salmon feed ingredients by volume are imported. As a result much of the global environmental impact from European aquaculture is also outside of Europe, including pressure on forage fisheries for marine ingredients, or reliance on crops that have been associated with deforestation such as soybean, as well known examples. The pressure has been to develop local and novel sources of ingredients that are low impact, often within “circular economies” using locally sourced by-products. However, despite initial promise, many of the novel ingredient industries have faced challenges around legislation, scalability, consistency, logistics, high costs and with sustainability credentials that have not met expectations. The Norwegian industry has specific targets around reducing reliance on imported feed ingredients whilst reducing environmental impacts so that all ingredients are from “sustainable sources” by 2030. In this paper we investigate the Life Cycle Assessment of the production of mussel protein ingredients for Norwegian salmon feed, made from locally produced mussels. Mussels are produced in seawater with no feed inputs and minimal intervention and therefore have a low environmental impact compared to some traditional ingredients. However, Norwegian conditions can sometimes lead to long culture periods and smaller mussels than in warmer locations. Nutritionally, deshelled ingredients have excellent properties, with high digestibility, protein levels of around 65%DM, suitable amino acid profile and low fat, making them an excellent candidate for salmon feed. Two main ingredients were tested; a mussel slurry that was not treated and a hydrolysed mussel silage.

Methods

Life Cycle Assessment data was collected from pilot facilities for the production of protein ingredients that were subsequently tested in nutrition trials. Data were collected on yields of mussels from pilot commercial production facilities in Hardangerfjord, fuel use and other servicing along with data on processing mussels into feed ingredients, including energy use, chemical inputs and yields of products. Performance of the mussel ingredients was tested in nutrition trials, from which the overall environmental impacts of salmon produced using the different ingredients could be assessed.

Results and discussion

Results are still being analysed and will be and will be provided, detailing LCA impacts of mussel production, ingredient production per tonne of ingredient and their performance in salmon diets from the nutrition trials. This section will be updated accordingly.

Acknowledgements

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RESTORING MARINE BIVALVE HABITATS – FOCUS ON BLUE MUSSELS *Mytilus edulis*

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Introduction

Globally, marine restoration has become an increasingly important measure to regenerate ecosystems, protect biodiversity, and support ocean sustainability. In Denmark, marine nature restoration is still a relatively new field, and significant experience and knowledge have been built up over the past 15–20 years, through the implementation of various research and citizen-based projects in Danish waters. These initiatives have primarily concentrated on establishing boulder reefs and eelgrass meadows, while the restoration of shellfish beds was only initiated in recent years.

In 2022, the Center for Marine Restoration (CMR) was founded. CMR is a Danish national centre that spans four institutions: Technical University of Denmark, University of Southern Denmark, Aarhus University, and The Limfjord Council (a local organisation of municipalities around the Limfjorden). The purpose of CMR is to ensure that marine nature restoration in Denmark is carried out on a knowledge-based foundation by collecting, mapping, and analysing existing and new knowledge, and by providing this expertise to local, municipal, and national stakeholders to support the successful implementation of different types of marine restoration projects.

Results

Along the shallow Danish coastlines, the current shellfish beds are dominated by blue mussel beds (*Mytilus edulis*), whereas horse mussels (*Modiolus modiolus*) and European flat oysters (*Ostrea edulis*) occur only sporadically, if at all. This presentation focuses on the restoration of blue mussel beds, outlining their historical national distribution and reviewing the key ecosystem functions they support. This is followed by an update on recent trends in international and national mussel populations, demonstrating that the natural formation of stable mussel beds is under pressure and needs active restoration in Denmark. Finally, the common CMR guidelines for restoring blue mussel beds will be introduced, together with updates on experiences from restoration efforts in Danish waters.

Acknowledgment

The Center for Marine Restoration is funded by the Velux Foundation and the Danish Ministry of Environment and Gender Equality.

RESISTANCE TO RAINBOW TROUT FRY SYNDROME FOR UNTREATED AND TREATED RAINBOW TROUT (*Oncorhynchus mykiss*) FAMILIES

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Introduction

Rainbow Trout Fry Syndrome (RTFS), caused by *Flavobacterium psychrophilum*, is a globally important disease in rainbow trout aquaculture, particularly affecting fry and juvenile fish with high mortality (Hesami et al., 2011; Muñoz-Atienza et al., 2019). For larger fish mortality rates are low, but morbidity rates can be high (Madsen & Dalsgaard, 1999; Nilsen et al., 2011). Its impact on production can be substantial, leading to significant economic losses in commercial aquaculture. No commercial vaccine is available, and disease control relies mainly on antibiotics, raising concerns about environmental impact and antimicrobial resistance. These challenges are especially significant in organic aquaculture, where treatment options are limited. EU targets for increased organic production and reduced antimicrobial use highlight the urgent need for alternative, preventive disease management strategies in trout farming. The heritable nature of resistance to RTFS is known. Today it is however unknown if disease resistance for treated fish can be considered the same trait as disease resistance for untreated fish in a natural outbreak situation.

Materials and Method

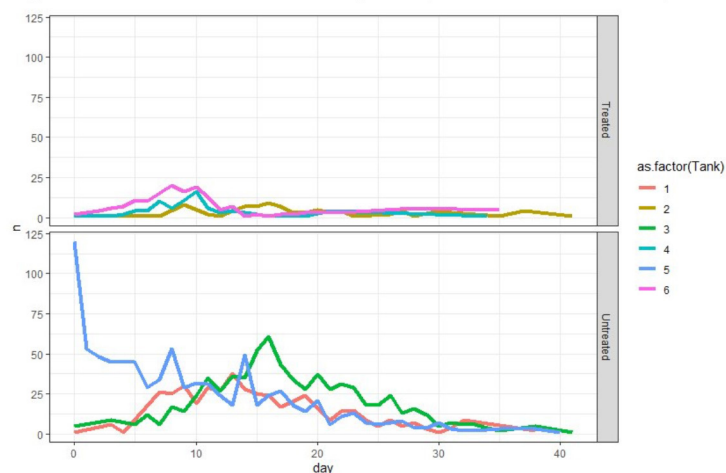
Rainbow trout (*Oncorhynchus mykiss*) from 210 full- and half-sib families were sampled at an organic farm in Denmark during a natural RTFS outbreak in 2025. Fish from each of the 210 families were stocked into three untreated and three untreated tanks. The presence of *Flavobacterium psychrophilum* was confirmed by a veterinarian through microscopic identification of the bacteria in blood samples. During the outbreak, deceased fish were sampled daily. Data collection of deceased fish included date of death, weight, length, and tissue samples for genotyping.

Results and discussion

Mortalities for each day for treated (1, 3, 5) and untreated tanks (2, 4, 6) are shown in Figure 1. The outbreak lasted on average 47 days, from mid-September to early November. Mortalities were on average 9% for treated fish and 54% for untreated fish. Further analysis will be done to estimate genetic correlations between treated and untreated fish.

This study provides insights into genetic components impacting resistance to RTSF in untreated and treated rainbow trout after a natural disease outbreak. These findings are important in order to know if selecting for disease resistance for treated fish will be effective for improving resistance in untreated fish.

Figure 1. Mortalities for treated (1, 3, 5) and untreated tanks (2, 4, 6).



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Acknowledgement

This work was part of the TROUTGANIC funded by the Green Development and Demonstration Programme (GUDP) under the Danish Ministry of Food, Agriculture and Fisheries, The project is part of the Organic RDD 8 programme, which is coordinated by ICROFS.

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FROM BEHAVIOURAL INDICATORS TO TRANSFORMER-BASED MODELLING: LEARNING FISH DYNAMICS FROM 3D SONAR DATA FOR INTELLIGENT FEEDING

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Feed inefficiency remains a major challenge in aquaculture, leading to economic loss and environmental impact through wasted feed and nutrient discharge. Current feeding practices rely on operator experience and camera observations, which provide limited coverage in large sea cages. In contrast, 3D multibeam sonar enables continuous, volumetric monitoring of fish populations, making it possible to observe behaviour across the entire cage.

Previous work [3] showed that combining 3D sonar with environmental sensing can extract useful behavioural indicators, such as vertical fish distribution and activity patterns linked to feeding. However, these approaches mainly rely on static or aggregated features and do not fully capture how behaviour evolves over time. Feeding behaviour is inherently dynamic, with gradual changes in fish distribution and activity.

In this study, we introduce FEAT (Fish Echo Activity Transformer), a transformer-based framework for modelling fish feeding behaviour directly from sonar data. Each sonar recording is represented as a sequence of depth-resolved acoustic frames, preserving how fish distribution changes over time (Fig. 2). This enables the use of sequence modelling to capture temporal behaviour patterns.

A complete processing pipeline is developed (Fig. 1), including cage masking, transformation of sonar data into compact depth–intensity profiles, and sequence modelling using a transformer encoder. The model is inspired by recent advances in sequence learning and adapted to continuous acoustic data.

The method follows a two-stage training strategy. First, a self-supervised pre-training stage uses masked frame reconstruction, where part of the input is removed and the model learns to reconstruct it from context. This allows the model to learn general patterns of fish activity from large amounts of unlabeled data. Second, the model is fine-tuned using labelled data to classify feeding and non-feeding sessions.

To ensure a fair evaluation, the dataset is split by calendar date, so the model is tested on unseen recording periods. The data consist of sessions with different lengths and sampling frequencies, reflecting different acquisition modes during feeding and non-feeding periods (Fig. 2). This introduces additional complexity, as recording protocol and feeding activity are partly correlated.

Evaluation on a held-out test set ($n = 261$ sessions) shows that the model captures meaningful behavioural signals. It achieves an AUC-ROC of 0.865 and a recall of 0.872, indicating strong ability to detect feeding events. Precision is lower (0.506), due to false positives (Fig. 3). This suggests that the model prioritizes detecting feeding events, which may be desirable in practice where missed feeding is more critical than false alarms.

Further analysis shows an important limitation: session length and sampling frequency are not independent of feeding activity. This means that part of the model's performance may be influenced by differences in data acquisition rather than behaviour alone. This highlights the need for improved data collection, ideally continuous acoustic recordings, to better isolate behavioural signals.

Despite this limitation, the proposed transformer-based approach is a clear step forward in modelling fish behaviour from acoustic data. By learning directly from temporal sequences, the model captures behavioural dynamics that are not represented in traditional feature-based methods. This opens new possibilities for understanding fish behaviour and detecting subtle changes related to feeding.

The results demonstrate the potential of transformer-based models for aquaculture monitoring and their role in future intelligent feeding systems. By enabling data-driven interpretation of fish behaviour at scale, such methods can support more efficient and sustainable feeding strategies.

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INTEGRATING SATELLITE OBSERVATIONS, FIELD MEASUREMENTS AND MODELLING TO MONITOR ENVIRONMENTAL IMPACTS OF MARINE FISH FARMING (SALUPA 1&2)

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Introduction and objective

Monitoring environmental impacts from marine fish farming is challenging in coastal waters, where farm-related effects interact with natural variability in water circulation, water-column structure and background water quality. Especially in the eutrophic Baltic Sea, distinguishing the effects of fish farming for monitoring purposes from elevated background nutrient concentrations and other sources of nutrient loading is particularly difficult. Satellite observations provide spatially extensive and repeatable information on surface conditions but attributing observed changes specifically to aquaculture requires comparison with field measurements and modelling. The work builds on SALUPA 1 (2024–2026), which developed and initially evaluated an integrated approach combining Earth observation, field monitoring and modelling to assess pressures on sensitive coastal habitats. The ongoing SALUPA 2 project (2026–2028) extends and further validates this approach for operational monitoring and environmental-impact assessment around marine fish farms in Finnish Baltic Sea coastal waters. The framework combines Sentinel-2 and Landsat products for chlorophyll-a and turbidity with satellite sea-surface-temperature observations. The 2024 field campaigns provided calibration and interpretation data through continuous flow-through surface observations, EXO profiles, laboratory-analysed water samples and acoustic Doppler current profiler measurements at three marine areas. In 2025, additional field campaigns were conducted at six selected marine farming areas, with sampling coordinated where possible with satellite overpasses. Measurements covered horizontal gradients and vertical water-column variability. Existing farms in contrasting hydrodynamic settings were additionally examined using the Finnish Coastal Nutrient Load Model (FICOS), with paired simulations with and without farm loading to estimate the farm contribution against background conditions.

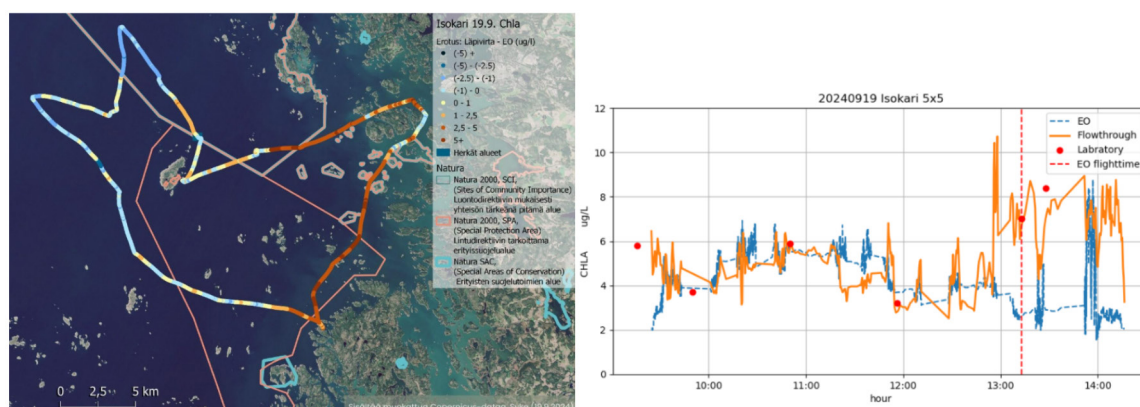


Figure 1. Comparison of chlorophyll-a (Chl-a) concentrations measured along the Isokari survey route on 19 September 2024. The panel on the right shows the temporal variability of Chl-a derived from EO observations (blue dashed line), flow-through measurements (orange line), and laboratory analyses (red points). The red dashed line indicates the EO acquisition time. The picture on the left presents the spatial distribution of Chl-a along the vessel transects, with colours representing concentration classes derived from EO data. Overall, the results show good agreement between the different observation methods at moderate concentrations, while larger discrepancies occur in areas with elevated Chl-a levels and strong spatial variability. The map highlights substantial heterogeneity in Chl-a concentrations across the study area, emphasizing the importance of combining spatial EO observations with in situ measurements for environmental monitoring.

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Preliminary results and conclusions

Preliminary comparisons from SALUPA 1 show that Earth-observation products reproduce relevant spatial and temporal water-quality patterns. In the 2024 field campaigns, satellite-derived turbidity corresponded well with calibrated flow-through observations calibrated with laboratory samples. However, eg. chlorophyll-a values showed substantial variability between satellite and on-site observations. FICOS simulations at two operating farms likewise indicated that modelled farm-related chlorophyll-a increments were generally small relative to the total concentration range, although their magnitude and dispersal differed between hydrodynamic settings. These findings show that a surface satellite signal alone cannot yet consistently establish aquaculture causality. Satellite observations should therefore as of yet complement rather than replace field monitoring. Their principal value is to extend observations around fixed sampling locations, increase seasonal variety, identify spatial patterns and support the targeting of field campaigns. Field measurements remain necessary for calibration, vertical context and confirmation, while hydrodynamic and nutrient modelling supports attribution of observed changes to farm loading rather than background variability. SALUPA 2 is using the expanded 2025 field dataset to refine cross-platform comparisons, uncertainty assessment and data-integration procedures for operational monitoring and environmental-impact assessment of marine fish farming.

Acknowledgment

The SALUPA 1 & 2 project was funded by the European Maritime, Fisheries and Aquaculture Fund. We thank the Kalavaltio project for valuable field observations.



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ASSISTED NET HOLE DETECTION IN FISH FARMS USING COMPUTER VISION

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Fish escapes are a persistent problem for the aquaculture industry, and most trace back to undetected net damage. A single cage can contain around 5,000 m² of net surface, which ROV (Remotely Operated Vehicle) pilots must inspect by simultaneously controlling the vehicle and visually scanning the net for extended periods in poor and variable visibility. This dual-task workload over long inspection runs creates substantial risk of overlooked damage, with serious ecological and economic consequences. Escaped fish can interact with wild populations and transmit disease [1], and escape risk is tightly regulated in major salmon-producing regions such as Norway, where authorities track containment performance [2].

Visibility during inspections is often reduced by low and varying lighting, turbidity, motion blur, and biofouling. Combined with the monotony of long inspection runs, these factors make lapses in attention likely, leaving damage undetected. While computer vision approaches to net damage detection have been explored [3], prior work has largely been validated on staged or controlled data such as pools, tanks, and nets that are not representative of operational conditions. Notable exceptions [4][5] evaluate on imagery from real fish cages but rely on traditional image processing and classical machine learning rather than modern object detection, and neither is validated within routine commercial inspection operations.

This work presents a YOLOv9-based detector framework [6] that runs in real time alongside the ROV pilot, flagging candidate net damage on the live video feed as a visual redundancy layer (Figure 2). Inference runs on topside hardware rather than onboard the ROV. Detections are associated across frames using ByteTrack [7] to stabilize alerts and suppress spurious single-frame detections during live review.

The model is trained and evaluated on in-situ inspection footage provided by Njord Aqua, an industry partner performing daily ROV inspections for the fish farm industry. Video was recorded at 2560x1440 resolution and 30 fps across inspections at multiple Norwegian salmon farms. Each inspection produced raw video alongside an inspection report documenting identified damage with timestamps, which served as ground truth for annotation. Train, validation, and test splits were drawn from distinct inspections and farms to avoid data leakage; split statistics are reported in Table 1. Underwater-specific augmentations were applied to address common imaging challenges such as suspended particles, motion blur, and light scattering.

The detector is evaluated on a held-out test set of 4,620 frames drawn from 6 inspections across 6 Norwegian salmon farms not seen during training. The test set covers variation in visibility, biofouling, and lighting representative of routine operations.

At the deployed operating point (confidence threshold 0.05), the model achieves a precision of 0.89 and recall of 0.36 on the test set, with mAP@0.5 (mean average precision at IoU 0.5) of 0.63 (Table 2). Relative to a baseline trained without underwater-specific augmentations, precision improves from 0.83 to 0.89 at comparable mAP, shifting the precision-recall balance toward fewer false positives per inspection. This means the detector is not strictly better, but better-suited for a pilot-verified workflow where false-positive rate directly affects operator trust. The optimal operating point is not obvious. A higher false-positive rate may be acceptable if it surfaces additional missed damage, since each detection is verified by the pilot.

The detector runs at 46 FPS at 640x640 input on NVIDIA RTX 3500, comfortably exceeding real-time requirements for integration with the live ROV video feed. Beyond quantitative performance, the system has been deployed as an assistive tool during live commercial inspections by Njord Aqua, where it has detected net damage that pilots did not see. Still, there are cases of damage flagged by the detector and missed by pilots, and damage identified by pilots but not the detector, showing that the model is not yet reliable on its own. This is precisely the condition under which the system adds value: the detector and the pilot fail on different cases, and the combination catches more than either alone.

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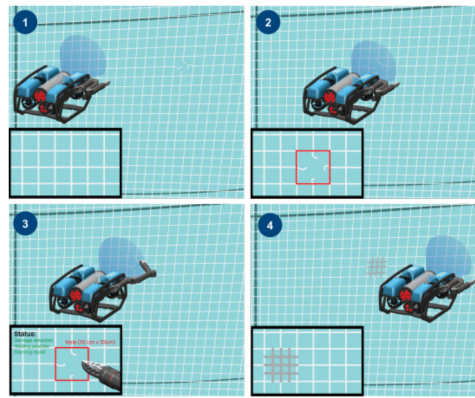


Figure 1: Steps during ROV inspections.

Model	P	R	mAP@0.5	FPS
Baseline	0.83	0.43	0.64	46
+ Underwater aug.	0.89	0.36	0.63	46

Table 1: Dataset splits.

Split	Images	Pos. rate	Inspections	Farms
Train	8,273	26.4%	16	12
Val	2,029	45.0%	9	6
Test	4,620	23.5%	6	6

Table 2: Test set performance at confidence threshold 0.05.

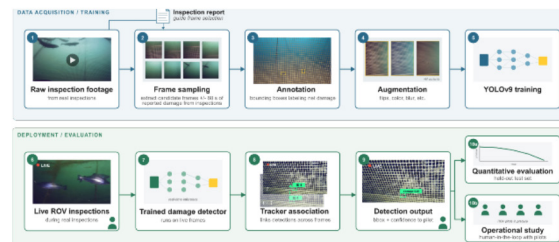


Fig. 2: Method abstract overview.

Acknowledgements

The work described has received funding from the Research Council of Norway. We are also grateful for the contribution of the industry partners, Njord Aqua and Måsøval.

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PHYLOGENOMICS OF *Knipowitschia* GOBIES: IMPLICATIONS FOR CONSERVATION AND TAXON DELIMITATION

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Introduction

Sand gobies of the *Knipowitschia* genus have evolved in coastal lagoons, estuaries, and freshwater ecosystems of the Adriatic basin and eastern Mediterranean (Tougard et al., 2021). While euryhaline lineages are mostly widespread, freshwater taxa are often endangered, endemic species with narrow distributions (Kovačić & Pallaoro, 2003; Miller, 2009). This pattern is likely resulting from repeated colonizations of freshwater habitats by euryhaline ancestors during the Last Glacial Maximum. Despite the isolated evolution of these lineages, many *Knipowitschia* gobies have developed similar morphological and ecological traits (Vanhove et al., 2012; Thacker & Gkenas, 2019; Tougard et al., 2021). Moreover, since the species diverged relatively recently, they remain genetically very similar. Thus, traditional mitochondrial markers have failed to resolve the evolutionary relationships among three key Adriatic species: *Knipowitschia panizzae*, *K. mrakovcici*, and *K. radovici* (Thacker & Gkenas, 2019; Tougard et al., 2021).

Materials and Methods

This research includes euryhaline and freshwater *Knipowitschia* species from the Adriatic basin and eastern Mediterranean, and a related freshwater species previously considered to belong to the same genus, *Orsinigobius croaticus*. For phylogenetic analyses, we aimed to obtain between two and four individuals per site. To further investigate complex relationships between the key Adriatic species, we strived to collect at least 10 individuals per locality for population genomic analyses. The extracted total genomic DNA was sent for whole-genome resequencing, aiming for an average coverage of approximately 20× per sample. We reconstructed phylogenetic relationships using IQTREE for a maximum-likelihood tree inference, ASTRAL for a coalescent-based species tree estimation, and the PHYLOCIRAPTOR workflow for a BUSCO-based ortholog set assessment. Finally, we used PCA to further examine the population structure of closely related Adriatic species.

Figure 1

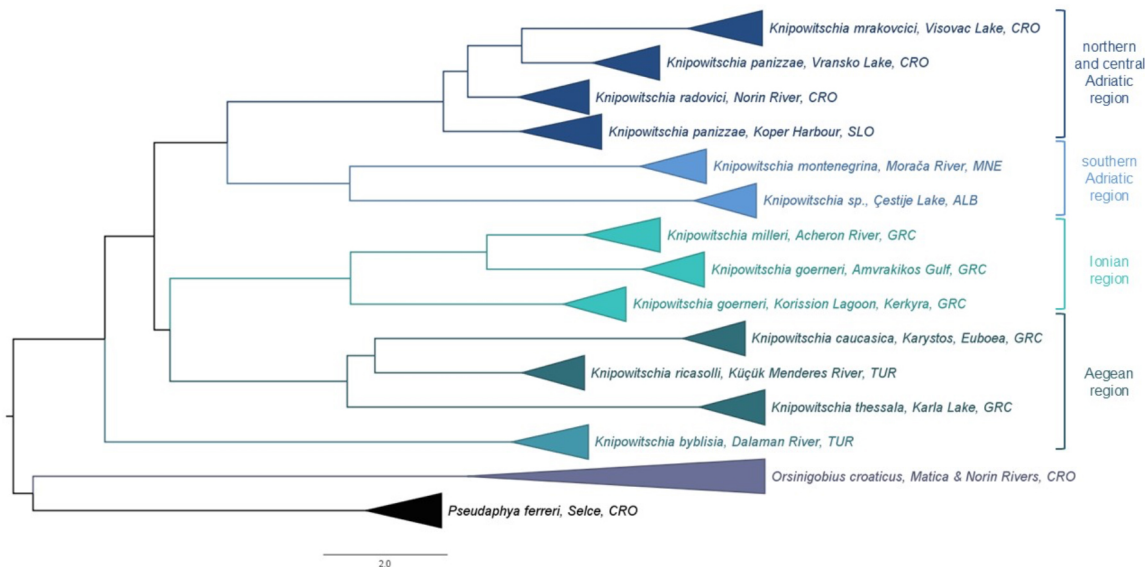


Figure 1. ASTRAL species tree inferred from IQ-TREE gene trees using whole-genome resequencing data. All nodes are supported by local posterior probabilities of 1.0. Scale bar represents branch lengths in coalescent units.

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Results & Discussion

We managed to collect a total of 123 samples from 16 localities for the 13 described species used in this study. The phylogenetic reconstruction suggests a strong phylogeographic structure, with lineages closely following the geographic distribution of investigated populations. The pronounced phylogenetic separation between the Vransko Lake and Koper Harbour populations of *K. panizzae* suggests that this taxon may represent a cryptic species complex requiring further taxonomic revision. A similar pattern is also observed in the Ionian region regarding *K. goerneri*, with the Amvrakikos Gulf population clustering with the sister species *K. milleri* rather than with the Korission Lagoon population. Furthermore, a recent divergence is observed among the closely related *Knipowitschia* species from the northern and central Adriatic region. Also, the PCA displayed a delineation between these populations, providing additional support for the presence of a cryptic species complex. Resolving this taxonomic uncertainty is the crucial first step towards the conservation of these taxa.

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DIETARY TRACE MINERAL SOURCES AFFECT PERFORMANCE AND HYPOXIA RESISTANCE OF JUVENILE *Penaeus vannamei*

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Introduction

Trace-mineral source can influence nutrient utilization, productive performance and the capacity of shrimp to withstand environmental stress. This study evaluated dietary source combinations of zinc (Zn), selenium (Se), copper (Cu), chromium (Cr) and magnesium (Mg) in juvenile *Penaeus vannamei* reared at low and optimal salinities, with emphasis on growth, feed efficiency and resistance to acute thermal and hypoxic stress.

Materials and methods

A total of 6,000 juveniles (2.28 ± 0.29 g) were stocked in 100 indoor 0.5 m³ tanks at 60 shrimp/tank (105 shrimp/m²) in a recirculating aquaculture system. Fifty tanks were maintained at 6 ppt and 50 at 21 ppt. Five mineral programs were evaluated with 10 replicate tanks/treatment/salinity: CTRL, containing inorganic Zn, Se, Cu, Cr and Mg; OLM1, containing organic Zn, Se, Cu and Cr with inorganic Mg; OLM2, containing organic Zn, Se, Cu, Cr and Mg; OLM3, containing inorganic Zn, Se, Cu and Cr with organic Mg; and REF, containing commercial methionine-based amino acid chelates of Zn, Se, Cu and Cr with inorganic Mg. At 0.50% dietary inclusion, all mineral premixes were formulated to target supplemental contributions to the finished diets of 120 mg Zn/kg, 0.40 mg Se/kg, 40.0 mg Cu/kg, 0.20 mg Cr/kg and 0.80 mg Mg/kg, irrespective of mineral source.

After a 6-day mineral-depletion phase, shrimp received the experimental diets for 70 days. Growth, survival, gained yield, feed intake and feed conversion ratio were determined. Performance analyses included 69 tanks after data screening. Thermal resistance was tested by exposing 10 shrimp/aquarium for 10 min to $18.1 \pm 0.05^\circ\text{C}$ or $34.0 \pm 0.05^\circ\text{C}$. Hypoxia resistance was evaluated with 20 shrimp/aquarium as dissolved oxygen declined to 0.7 ± 0.1 mg/L over 60 min, followed by a 24-h recovery period. Performance variables were analyzed by one- or two-way analysis of variance followed by Tukey's test, whereas stress responses were analyzed using Kruskal–Wallis and pairwise tests ($p < 0.05$).

Results and discussion

Survival remained high (93.6–97.6%). At 6 ppt, final body weight did not differ among diets ($p = 0.114$). At 21 ppt, OLM3 produced the highest final body weight (17.08 ± 1.13 g) compared with CTRL (15.77 ± 1.46 g), an 8.3% increase ($p < 0.0001$). Across salinities, OLM3 also produced greater gained yield than CTRL (1,478 versus 1,332 g/m²; $p = 0.037$) and the lowest feed conversion ratio (1.78 versus 1.92 in CTRL; diet effect $p = 0.015$), representing an improvement of approximately 7–8%. No diet $\times$ salinity interaction was detected for these variables ($p > 0.05$).

A dietary Mg requirement of 2.60–3.46 g Mg/kg diet has been reported for optimal growth of *P. vannamei* under low-salinity conditions (2 g/L), providing relevant nutritional context for evaluating Mg-source strategies (Cheng et al., 2005). OLM3 is particularly informative because it retained inorganic Zn, Se, Cu and Cr, as in CTRL, while replacing inorganic Mg with an organic Mg source. Its superior performance is therefore consistent with a potential benefit of organic Mg when used within an otherwise inorganic trace-mineral program. However, OLM2 also contained organic Mg and did not show a comparable performance advantage, indicating that the response was dependent on the overall mineral-source combination rather than Mg source alone. The present study was not designed to establish a dietary Mg requirement.

Thermal-challenge mortality was not significantly affected by diet ($p > 0.05$), although several supplemented treatments showed numerically lower mortality. Hypoxia produced a clear dietary response. At 60 min, when dissolved oxygen reached 0.7 ± 0.1 mg/L, mortality was $48.3 \pm 4.4\%$ in CTRL versus $6.7 \pm 7.6\%$, $18.3 \pm 12.6\%$, $13.3 \pm 2.9\%$ and $21.7 \pm 7.6\%$ in OLM1, OLM2, OLM3 and REF, respectively ($p = 0.0414$). After 24 h recovery, mortality was $16.7 \pm 5.8\%$ in CTRL, 0% in OLM1, OLM3 and REF, and $1.7 \pm 2.9\%$ in OLM2 ($p = 0.0182$). OLM1 provided the strongest protection during severe oxygen depletion, while all supplemented mineral programs improved post-hypoxia survival relative to CTRL.

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Conclusions

Trace-mineral source strategy affected productive performance and hypoxia resistance in juvenile *P. vannamei*. OLM3 was the most effective program for final body weight, biomass yield and feed efficiency. The direct contrast between CTRL and OLM3 is consistent with a potential advantage of replacing inorganic Mg with organic Mg when Zn, Se, Cu and Cr are supplied from inorganic sources; however, the response across all treatments indicates that performance depended on the complete mineral-source combination rather than Mg source alone. OLM1 provided the strongest resistance to acute hypoxia, whereas thermal-stress responses did not differ statistically among diets. These findings demonstrate that selection of trace-mineral sources can produce distinct effects on production efficiency and resilience to severe oxygen depletion under low- and optimal-salinity culture conditions.

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ADVANCING MARINE AQUACULTURE IN THE UNITED STATES: AN UPDATE ON STRATEGIC, NATIONAL INITIATIVES

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Although the United States is a global leader in aquaculture science and technology, our production lags relative to much of the world and to our potential. Executive Orders “Promoting American Seafood Competitiveness and Economic Growth” and “Restoring American Seafood Competitiveness” aim to increase U.S. seafood production through, in part, advancing domestic aquaculture. National Oceanic and Atmospheric Administration (NOAA) is charged with leading these efforts.

NOAA is taking a multifaceted approach to support aquaculture, including through spatial planning, robust environmental reviews, and research investments. A key part of this strategy is the identification of Aquaculture Opportunity Areas (AOAs). By proactively evaluating broad regions for their environmental, economic, and social suitability, NOAA significantly advances the siting process for commercial aquaculture while minimizing conflicts with protected species and other ocean users. 13 AOAs have already been identified in two regions of the country, others are under development in Alaska, and NOAA is in the process of determining where to launch a new AOA effort.

In addition, NOAA recently made a historic scientific investment by establishing a nation-wide, long-term aquaculture research partnership - the Cooperative Institute Fostering Aquaculture Research and Markets (CIFARM). Through CIFARM, NOAA will collaborate with numerous academic institutes, industry partners, and non-governmental experts to address critical challenges using artificial intelligence, engineering innovation, and seafood market research.

Together, these and other coordinated initiatives comprise a comprehensive national strategy to increase seafood competitiveness, reduce reliance on imported seafood, and support economic opportunities across seafood supply chains.

EVIDENCE FOR THE EVOLUTION OF RESISTANCE TO NON-CHEMICAL PARASITE CONTROLS: MULTIGENERATIONAL SELECTION REVEALS HERITABLE DEEP-SWIMMING BEHAVIOUR IN SALMON LICE

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Introduction and methods

Salmon lice (*Lepeophtheirus salmonis*) pose a major challenge to the sustainability of salmon aquaculture due to their capacity to rapidly evolve resistance to parasite control methods. As the effectiveness of chemical treatments has declined, the industry has increasingly relied on preventive strategies to limit initial infections. One such approach is depth-based farming, where fish are held deeper in the water column using submerged cages to reduce exposure to infective stages that concentrate near the surface. Preventative management strategies can impose sustained selection on parasite populations, but evolutionary responses depend on both the strength of selection and the availability of heritable variation. While resistance to chemical treatments is well documented, less is known about whether parasite behaviours can evolve in response to non-chemical control methods.

In this study, we investigated whether vertical swimming behaviour in salmon lice larvae is heritable and responsive to selection. First, we sampled 122 adult female lice carrying egg strings from commercial farms using either standard cages (0–20 m) or submerged cages (20–40 m). The first-generation larvae were reared under controlled conditions, and the vertical positioning of 11,291 copepodid larvae was assessed in pressure columns simulating a depth of 10 m. In a complementary multi-generational experiment, larvae originating from approximately 500 lice collected from five farms were subjected to directional selection for surface swimming, bottom swimming, or random selection across three generations.

Results and discussion

Larval vertical distribution was significantly influenced by parental environment, with a strong interaction between depth behaviour and cage type ($\chi^2 = 278.85$, $df = 1$, $p < 0.001$). Larvae from standard cages showed a greater tendency to ascend (35% vs. 23%) and were less likely to sink (19% vs. 27%) compared to larvae from submerged cages, indicating that vertical swimming behaviour is at least partly heritable. Experimental selection further demonstrated that this behaviour can evolve: surface-selected larvae increasingly occupied upper layers, whereas bottom-selected larvae showed consistent shifts towards deeper swimming over successive generations. By the third generation, bottom-selected larvae were 11% more likely to occur at depth than the founding population.

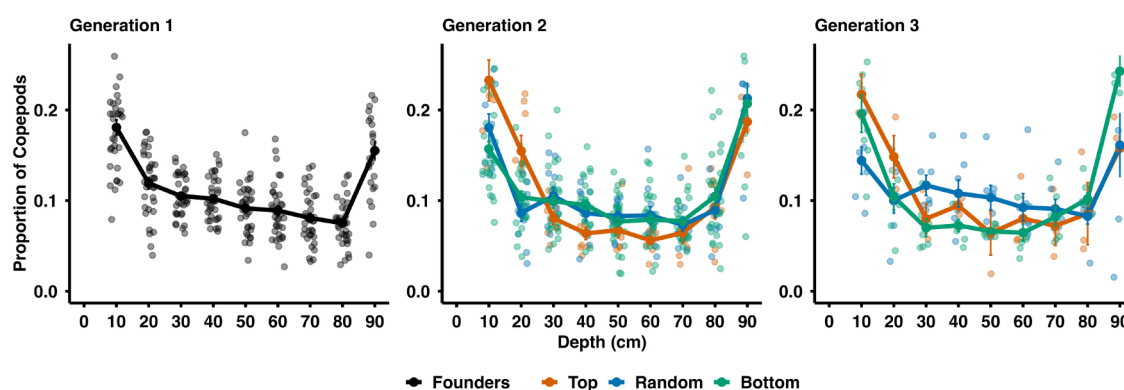


Figure 1. Mean proportion of copepodids across 10–90 cm depth by generation. (A) Founding generation (F_1), before selection. (B) Second generation (F_2), showing three lines selected for vertical swimming behaviour — top 10% (red), bottom 10% (green), and random (purple). (C) Third generation (F_3), following another round of selection.

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Together, these findings provide evidence that vertical swimming behaviour in *L. salmonis* is both heritable and evolutionarily responsive to sustained selection. This reveals a potential pathway for adaptation to depth-based farming strategies. Although the rate of behavioural change appears gradual, the continued use of submerged cages may select for deeper-dwelling lice over time. These results highlight the importance of incorporating evolutionary principles into parasite management, suggesting that the long-term sustainability of submerged systems will depend on mitigating selection pressure, for example through the maintenance of refugia.

Acknowledgment

We thank the Institute of Marine Research for assistance with the initial lice collections, and the technical staff, 2025 summer interns, and apprentices at Matre for their support with infection procedures and maintaining lice across generations. We are grateful to Velimir Nola and Kris Oldham for help with lice collection, and to Stein Carlos Lopez Fyllingsnes, Torfinn Aga and Martin Matre for maintaining the pressure columns. We also acknowledge technical support with fish husbandry, particularly Thea Svendsen and Tone Vågseth for assistance with fish sedation.

GENOMIC AND MICROBIOME CONTRIBUTIONS TO VARIATION IN OMEGA-3 FATTY ACID TRAITS IN ATLANTIC SALMON (*Salmo salar*) DURING THE SALTWATER PRODUCTION PHASE

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Background

Variation in omega-3 fatty acid traits in Atlantic salmon (*Salmo salar*) is jointly shaped by host genomic architecture and gut microbiome composition, yet their relative contributions remain largely unquantified under plant-based feeding systems. The replacement of marine ingredients with plant-based alternatives in aquaculture has reduced the levels of long-chain omega-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). This poses a major challenge for nutritional quality and sustainability (Skavang *et al.*, 2024). While host genetics and diet are established determinants of fatty acid composition, the gut microbiome is increasingly recognized as a key regulator of nutrient metabolism, including lipid digestion, absorption, and metabolic processing (Bhat *et al.*, 2023; Gajardo *et al.*, 2016). Gut microbiome composition in Atlantic salmon is influenced by environmental and dietary factors, but these alone do not fully explain variation, suggesting a role for host-microbiome interactions (Dehler *et al.*, 2017; Webster *et al.*, 2020). Despite growing interest in these interactions, the extent to which gut microbial taxa contributes to omega-3 phenotypic variation remains poorly quantified. This study aims to quantify the contribution of gut microbial taxa to omega-3 traits and to identify host-microbiome associations underlying variation in fatty acid composition in Atlantic salmon.

Materials and Methods

A total of 425 Atlantic salmon (*Salmo salar*) from 30 full-sib families (AquaGen, Norway) were reared under standard aquaculture conditions and sampled at a mean body weight of 1,301.5 g. Distal intestinal digesta were collected postmortem for microbiome analysis. Fish were genotyped using a 56K SNP array, and after quality control (call rate ≥ 0.90 , MAF ≥ 0.01 , HWE $P > 1 \times 10^{-7}$), 47,792 SNPs were used to construct the genomic relationship matrix. Genomic DNA was extracted using the TGuide Smart Envir-DNA Kit and quantified with the Qubit dsDNA HS Assay Kit. V3-V4 region of 16S rRNA gene was amplified using primers 341F/806R in a one-step indexed PCR (10 μ l reaction; 21 cycles: 95 °C 30 s, 50 °C 30 s, 72 °C 40 s). Amplicons were purified (AMPure XP beads), pooled equimolarly, and sequenced on Illumina NovaSeq 6000 platform (PE250). Reads were filtered using *fastp*, trimmed with *cutadapt*, and processed with DADA2 (truncLen = 240/220; maxEE = 2). Taxonomy was assigned using SILVA v138.1. Gut microbiome data were integrated with host phenotypes, including EPA, DHA, EPA+DHA, ALA, OA, MUFA, SFA, and omega-6 fatty acids. Associations between microbial taxa and omega-3 traits were tested using linear mixed models including fixed effects (batch, tank, sex, weight, fat) and random effects (tank and host genetic structure). Microbiome contributions to phenotypic variance (m^2) were estimated across models. GWAS and microbiome GWAS (mGWAS) were conducted to identify host genetic and microbial associations. Microbial diversity metrics (alpha diversity, Faith's phylogenetic diversity, and UniFrac-based beta diversity) were computed using *vegan* and *picante*, with PCoA1 used as a microbial trait. Heritability (h^2) of prevalent ASVs ($n = 243$) was estimated using genomic linear mixed models (ASReml v4.2), and significance was assessed using likelihood ratio tests ($P < 0.05$).

Results

Variance partitioning showed that microbiome, host genomic, and residual effects contributed differentially across fatty acid traits (Fig. 1A). Microbiome contributions to EPA variation ranged from 11-16% ($m^2 = 0.123$ -0.157) depending on model specification, with increased explanatory power when fat content was included. Batch effects contributed significantly to variation, indicating strong environmental or technical influences. For DHA, microbiome contributions were lower but increased substantially when fat was included, from 3-5% to 9-10% (m^2 up to 0.104), suggesting indirect relationships mediated through lipid metabolism. Across lipid classes, microbiome effects were clearly phenotype-specific, explaining 12.3% of variance in EPA, 10.6% in DHA, and 11.7% in EPA+DHA. Moderate contributions were observed for MUFA (7.5%), OA (8.0%), and ALA (4.4%), whereas negligible contributions were detected for SFA (0.0%), omega-6 (0.0%), and PUFA (0.7%), indicating selective microbial influence on lipid metabolism. Host genomic effects varied across traits and were generally larger than microbiome contributions for several lipid classes (Fig. 1A). Genome-wide association analysis for EPA identified multiple loci exceeding suggestive and genome-wide significance thresholds (Fig. 1B), indicating a genetic basis for EPA variation. Several SNPs showed strong association signals across chromosomes, highlighting candidate genomic regions contributing to variation in omega-3 traits. Several microbial taxa exhibited moderate to high

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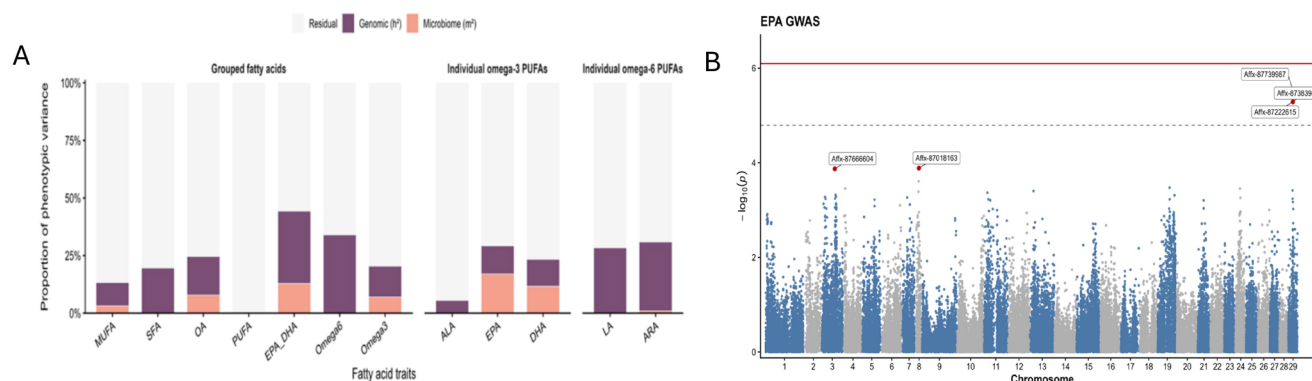


Figure 1. Genomic and microbiome contributions to variation in fatty acid traits and genome-wide association signals for EPA in Atlantic salmon. (A) Proportion of phenotypic variance explained by residual (grey), host genomic (purple), and microbiome (orange) effects across grouped fatty acids and individual lipid traits. (B) Genome-wide association results for EPA showing $-\log_{10}(p)$ values across chromosomes. The red horizontal line indicates the genome-wide significance threshold, and the dashed line indicates the suggestive significance level. Highlighted loci represent SNPs with the strongest associations with EPA variation.

heritability, with top ASVs showing h^2 ranging from 0.48 to 0.87 ($p < 10^{-3} - 10^{-8}$), demonstrating strong host genetic control over specific components of the gut microbiome. Association analyses (GWAS and mGWAS) further identified significant links between microbial taxa and omega-3 traits, including EPA, DHA, EPA+DHA, and ALA, supporting the role of host-microbiome interactions in shaping fatty acid composition.

Discussion

These findings demonstrate that the gut microbiome contributes a modest but significant and phenotype-specific proportion of variation in omega-3 traits in Atlantic salmon, with stronger effects observed for long-chain omega-3 fatty acids (EPA and DHA) than for other lipid classes. The progressive increase in microbiome contribution after accounting for fat indicates that microbial effects are conditional and mediated through host lipid metabolism, consistent with known microbial roles in short-chain fatty acid production, bile acid transformation, and modulation of host lipid absorption and elongation-desaturation pathways (Bhat *et al.*, 2023). The absence of detectable microbiome effects for DHA under baseline models, followed by their emergence after inclusion of fat, further supports a metabolism-dependent mechanism of microbial influence. These results align with previous findings that gut microbiota influences metabolic efficiency and nutrient utilization in Atlantic salmon (Gajardo *et al.*, 2016). The identification of highly heritable microbial taxa up to 0.15 indicates substantial host genomic control over specific microbiome components, supporting previous evidence that host genetics can shape microbial composition (Difford *et al.*, 2018; Wen *et al.*, 2021). These results reveal biologically meaningful host-microbiome interactions influencing omega-3 metabolism and suggest the potential to integrate microbiome information into breeding to improve omega-3 deposition, nutritional quality, and resource efficiency in farmed Atlantic salmon.

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IS IMTA JUST A BUZZWORD FOR POLYCULTURE? REDEFINING THE IMTA CONCEPT

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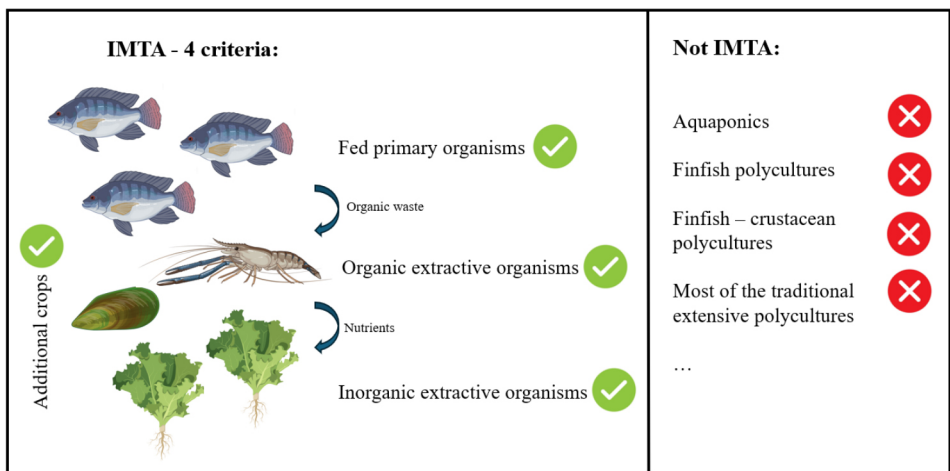
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Clear definitions are essential for understanding rules, concepts, and phenomena, preventing confusion with other terms, enabling clear communication, and supporting progress in any field. In aquaculture, there are many types of technical and biological solutions that try to improve productivity or tackle environmental problems related to faeces and uneaten feed. Particular attention has been given to a specific form of polyculture - integrated multi-trophic aquaculture (IMTA), which treats wastes from primary fed organisms using organic and inorganic extractive organisms that can be utilised as additional crops. Despite its clear conceptual foundation, IMTA is increasingly used as a catch-all term for almost any form of polyculture. Our study explains why an oversimplified definition of IMTA as a system in which the waste of one organism is utilised by another results in IMTA being practically the same as polyculture, thereby questioning the need for this new concept with high popularity. Based on a reassessment of the earliest IMTA literature, we propose four criteria that must be fulfilled for a system to qualify as IMTA *sensu stricto*. They give a clear distinction between true IMTA systems and other polycultures, such as aquaponics, fish-crustacean polycultures and others, which we systematically organise and discuss. We also systematically analyse 769 indexed articles mentioning IMTA, checking their compliance with the criteria. By clarifying IMTA terminology in line with its original concept, this paper provides a framework that supports more precise classification, comparison, and development of sustainable aquaculture systems.

Methods

We conducted a systematic review of peer-reviewed IMTA studies using Scopus and Web of Science, searching titles, abstracts, and keywords for “integrated multi-trophic aquaculture”, “integrated multitrophic aquaculture”, or “IMTA” AND “aquaculture”. After filtering for English-language research articles, removing duplicates and reviews, 769 publications remained for analysis. Taxa mentioned in each document were automatically identified using custom R code and an extensive taxonomic dictionary, standardizing organism names to unified taxonomic groups. Organisms were then classified into primary cultured species, organic extractive species, and inorganic extractive species, while feeding status was assigned based on taxonomic and contextual rules. The automated workflow was validated on a random subset of 50 articles, confirming high consistency in taxa recognition and functional classification.

Graphical abstract:



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Figure 1

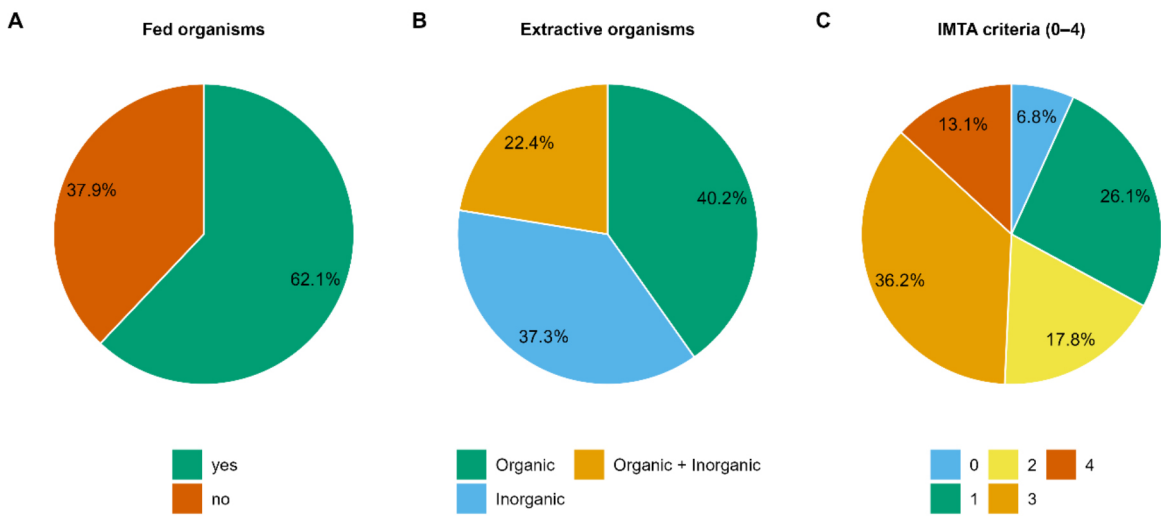


Figure 1: IMTA-related articles and their proportions of using fed organisms (A), secondary extractive organisms (B) and fulfilment of IMTA 4 criteria (C).

Figure 2

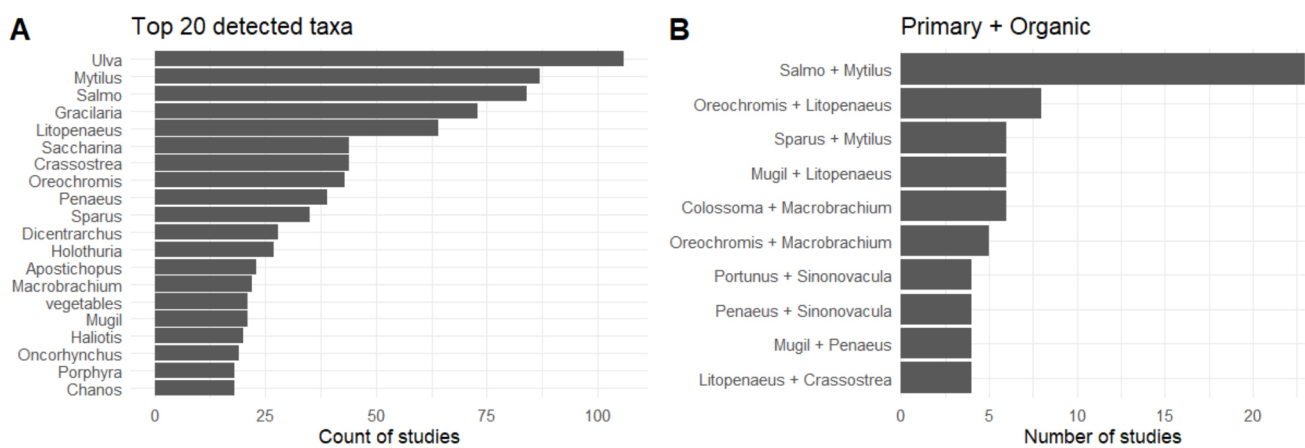


Figure 3: The most frequently used taxa in IMTA-indexed research: overall (A) and in combinations of primary raised species and organic extractive species (B).

ESTIMATION OF DEMOGRAPHIC HISTORY FOR ATLANTIC SALMON USING GENOMIC DATA

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Background

Atlantic salmon (*Salmo salar*) is one of the most economically important aquaculture species and a major contributor to the global supply of high-quality protein. As salmon breeding programmes have advanced from pedigree-based selection to the use of genome-wide marker data, managing genetic diversity has become increasingly important for sustaining long-term genetic gain and breeding programme sustainability (Ødegård et al., 2014; Tsai et al., 2017). Reconstructing demographic history in salmon is challenging, since estimates of effective population size (N_e) can be skewed due to the anadromous life cycle, population structure, varying recombination rates, and the whole genome duplication event that salmon genomes underwent in the distant past (Palstra et al., 2009; Rougemont et al., 2023). Including demography, recombination or genome duplication, may impact the efficacy of natural selection and the burden of deleterious mutations, is a central question in evolutionary biology and genetics. In this study, we show that key evolutionary processes, including variations in N_e . Estimating historical N_e more accurately can help better understand current patterns of diversity as observed in farmed and wild populations. Estimating N_e is crucial for preserving genetic diversity and can inform selective breeding programmes to improve their efficiency (Houston et al., 2020).

Aims

The aims of this study were to reconstruct the demographic history of Atlantic salmon across recent and ancient timescales using linkage disequilibrium (GONE software) and Pairwise Sequentially Markovian Coalescent (PSMC) methodologies. Further, sensitivity analyses were conducted to test how genomic regions retaining duplicated loci after the rediploidisation process (residual tetraploidy) and regional differences in recombination might affect N_e estimates.

Materials and Methods

Whole-genome sequence data from 74 Atlantic salmon were analysed to study their demographic history in recent and distant evolutionary periods. The dataset used included wild populations from Canada (30 samples) and Norway (26), together with a farmed population from Scotland (18). To assess genetic variation and population structure among populations, principal component analysis (PCA) and pairwise F_{ST} were estimated in PLINK v1.90b7.2, while ancestry proportions were inferred using ADMIXTURE v1.3.0 (Liu et al., 2020). Recent N_e changes (1 to 1,000 years) were estimated using GONE (Santiago et al., 2020) drops, and expansions. We develop a theoretical and computational framework to infer the demographic history of a population within the past 100 generations from the observed spectrum of linkage disequilibrium (LD). For ancient demographic history (1,000 to 1,000,000 years ago), the PSMC method was used (Li & Durbin, 2011) Korean, European and Yoruban (West African). Sensitivity analyses were performed by excluding genome regions showing evidence of residual tetrasomy, including pericentromeric regions, and by restricting analysis to uniquely mapped regions.

Results

PCA showed that the Canadian Atlantic salmon samples were more distinct compared to the European Atlantic salmon, with Scottish and Norwegian salmon being more similar to each other. The ADMIXTURE analysis confirmed a mixed ancestry between Scottish and Norwegian populations. For ancient history, PSMC showed similar demographic trends across populations, with N_e declining from approximately 100,000 one million years ago to approximately 5,000 by 7,000 years ago, followed by varying peaks across populations towards the recent end of the trajectory. In contrast for recent history, GONE showed different regional magnitudes; Norway and Scotland had higher N_e than the Canadian samples, before they declined sharply towards the present to values close to 100, whereas the Canadian population retained higher N_e of ~ 1000 . Sensitivity analyses did not substantially alter these historical N_e patterns, indicating that estimates are robust.

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Discussions and Conclusions

This analysis provides a framework to combine recent and more ancient history estimation methodologies to obtain robust historical trajectories of N_e . Our results showed a clear regional differentiation between North American and European Atlantic salmon, as expected, and less differentiation between farmed Scottish and wild Norwegian populations. This can be explained by the development of modern breeding programmes in Europe, where founder populations often had a common origin. This is likely to have resulted in lower genetic divergence between them. Although it was shown that all populations shared broadly similar ancient N_e patterns, more recent demographic inference varies by geographical region. The observed demographic patterns remained stable even when accounting for residual tetraploidy and varying recombination rates. These findings provide a robust overview of Atlantic salmon recent and ancient demographic history. This work contributes useful insights into the processes shaping Atlantic salmon diversity and effective population size, offering essential context for breeding programme strategies and the management of diversity in farmed populations..

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A PRELIMINARY INVESTIGATION INTO THE POSSIBILITY OF DOMESTICATION OF SOLAFUNMI (*Sierrathrissa leonensis*) AS AN AQUARIUM FISH FOR ORNAMENTAL PURPOSE

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The research was done to determine the possibility of domestication of a *Sierrathrissa leonensis* as an Aquarium fish. Aquarium fish is a special featured, highly valued aquatic organism and mostly smallish in size, viewed within a confined environment mostly for healing, aesthetic and recreational purposes. *Sierrathrissa leonensis* given a trade name Solafunmi is a freshwater fish - commonly called Clupeid (English: West Africa pygmy herring). It was harvested in schools from a drying stream at Gbaiko-Bosso, Minna, Nigeria using scoop and trap nets, then transported in freshwater using a 25 litres partially opened jerry-can. The fish had pale to brown colour in natural habitat as at the time it was harvested.. Vitamin C was administered as an anti-stress and acclimatised for three days under room condition. Twenty fish was introduced into 10 litres of freshwater in glass Aquarium in triplicate without aerator, commercial formulated pelletized feed (40 % crude protein) was administered and accepted by the fish under these average (range) conditions (Water: pH 6.72 (5.9 - 7.5), Temperature-18.6 °C (16 – 21.5 °C), Dissolved Oxygen- 6.27mg/l¹(5.93-6.58mg/l¹), Conductivity- 309.667µs/cm (173.3 - 370.5µs/cm), Room Temperature- 23.9 °C (22 – 27.5 °C)) and high survival rate of 18.67 (18 - 19), that is 93 35 %₀ was recorded with the fish remaining very active, moving in groups and non-aggressive. The fish is very hardy, thus, highly recommended as an Aquarium fish. Further researches needed on its biology. to make it a better Aquarium fish especially to improve on phenotypic features.

Table : Means and range of water quality parameters, survival and mortality

Parameters	Ti	Tii	Tiii	Mean (Range)
Water volume (Litres)	10	10	10	10
Number stocked	20	20	20	20
Survival	19	18	19	18.67 (18-19)
Mortality	01	02	01	1.33 (1-2)
pH	5.9 – 7.3	5.8 – 7.2	6.6 – 7.5	6.72 (5.9-7.5)
Dissolved Oxygen(mg/l)	(6.04mg/l ¹ - 6.58mg/l ¹),	(5.93mg/l ¹ - 6.50mg/l ¹),	(6.04mg/l ¹ - 6.50mg/l ¹),	6.27mg/l ¹ (5.93mg/l ¹ -6.58mg/l ¹)
Water Temperature (°C)	16 – 21.5 °C	16 – 21.5 °C	16 – 21.5 °C	18.6 °C (16 – 21.5 °C).
Room Temperature (°C)	22 – 27.5 °C	22 – 27.5 °C	22 – 27.5 °C	23.9 °C (22 – 27.5 °C)
Conductivity	263.3- 365µs	266.4- 370.5µs	173.3-320µs	309.667(173.3-370.5µs)

SELECTIVE BREEDING PROGRAMS FOR BOOSTING ORGANIC FISH FARMING

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Introduction and project objectives

One of the main obstacles to increase production in the European aquaculture sector today is the high mortality and welfare challenges associated with diseases in conventional farming systems. Conventional farming is also challenged by high CO₂-emissions from feed production and environmental pollution from e.g. feed residues, with negative impacts on climate, eutrophication and wild biodiversity^{1,2,3,4}. Thus, further growth of the sector requires a green transition and stronger actions on improving fish welfare. Organic aquaculture may play a major role, as stressed in the European Green Deal⁵ and Farm to Fork Strategy⁶.

To our knowledge, only a few breeding programs currently fully meet organic standards in European finfish aquaculture. The current low demand and income from sales of organic juveniles makes the sale of organic seed to fish farmers less profitable for breeding actors. It is therefore necessary to get further insights on how to implement cost-effective breeding programs for organic aquaculture.

The overall objective of the SelectOrganic project is to develop selective breeding programs for the organic farming of European seabass, gilthead seabream, rainbow trout and Atlantic salmon, respecting high animal welfare requirements and aiming at improving species resilience, disease resistance, product quality and resource efficiency. Furthermore, we aim for fish utilizing as much alternative feed materials as possible.

Organisation of project work

The critical areas that SelectOrganic will push beyond the state of the art are: i) advancing a broad set of methodologies from defining breeding goals for organic aquaculture to include non-market values of public goods (e.g. low environmental impact), ii) phenotyping and validating fish health, welfare and stress resilience using conventional welfare indicators and innovative sensor technology, and iii) innovative alternative feed materials, reproductive strategies and breeding designs. Finally, new knowledge, methods, technologies and breeding strategies will be utilized and integrated into holistic plans for breeding programs for organic aquaculture as shown in Figure 1. Socioeconomic studies on market dynamics, fish welfare, environmental valuations and regulatory frameworks, together with breeding strategies involving a broad range of actors, will ensure realistic and successful implementation. Hence, we foresee substantial impact and expanded organic aquaculture in Europe.

Results and expected Impact

Preliminary results from a review and qualified inventory of gaps and challenges faced by organic selection programs will be presented. This will include prioritized selected traits of economic and welfare importance for improvement in selective breeding programs for organic fish, together with an assessment of their relevance and applicability within the European organic regulatory framework.

All project deliverables will drive research and innovation-based development by increasing genetic gain in growth, feed efficiency using alternative feed materials, product quality and survival from bacterial infections and heat waves. We expect to enhance competitiveness and growth of the aquaculture industry and stimulate consumer demand for, and sales of seafood.

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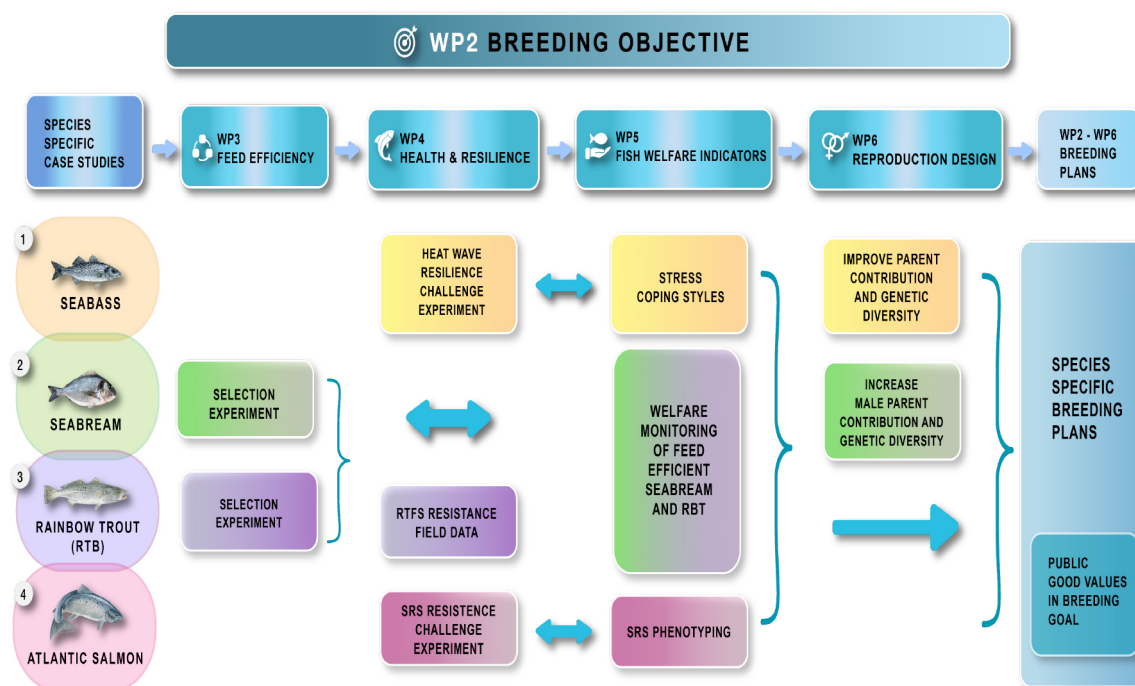


Figure 1 Overall technical workplan (WP2-WP6) for SelectOrganic

Acknowledgment

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TURNING AQUACULTURE EFFLUENTS INTO MICROALGAL BIOMASS: PILOT-SCALE CULTIVATION OF *Nannochloropsis oceanica* IN FLATFISH RAS EFFLUENT

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Introduction

Recirculating aquaculture systems (RAS) improve water-use efficiency and support the sustainable intensification of aquaculture, while generating nutrient-rich effluents that require appropriate management (1). Cultivating marine microalgae in these effluents offers an opportunity to recover residual nutrients and convert them into valuable biomass for aquaculture applications, supporting a circular bioeconomy strategy and resource-efficient production systems (1,2). Exploring cultivation strategies that promote bioremediation, efficient nutrient utilisation and biomass production is an important step towards the valorisation of RAS effluents (1). This study evaluated the cultivation of *Nannochloropsis oceanica* in treated RAS effluent from flatfish industrial production and investigated the effect of different nutrient supplementation strategies on culture performance and nutrient utilisation.

Materials and Methods

Pilot-scale cultivation of *Nannochloropsis oceanica* was performed at an industrial microalgae production facility in southern Portugal during winter in outdoor 100L flat-panel photobioreactors (FP-PBR). Four experimental treatments were established according to the nutrient supplementation strategy, with one FP-PBR per treatment: (Control) seawater supplemented with NutriBloom®; (Effluent) effluent without nutrient supplementation; (Effluent+nitrate) effluent supplemented with nitrate; and (Effluent+NutriBloom) effluent supplemented with NutriBloom®. Seawater routinely used for microalgae production at Necton following ultrafiltration and UV treatment was used as the control. RAS effluent was collected from a Senegalese sole (*Solea senegalensis*) grow-out production. Prior to cultivation, the RAS effluent was sequentially pre-filtered (50, 5 and 0.2 μm), and both the effluent and control seawater were chemically treated with sodium hypochlorite (1 mL L⁻¹ for 30 min), followed by sodium thiosulphate neutralisation (0.5 mL L⁻¹ for 5 min) and hydrogen peroxide (52.6 μL L⁻¹ for 30 min). Nutrient supplementation targeted the standard cultivation conditions for *N. oceanica* (4 mM nitrate and 0.2 mM phosphate). Nitrate and phosphate concentrations (mM) were determined before and after chemical treatment, following nutrient supplementation, and throughout cultivation. Culture performance was monitored through daily optical density (540 nm) measurements, and biomass concentration (g L⁻¹) was estimated using a previously established calibration equation.

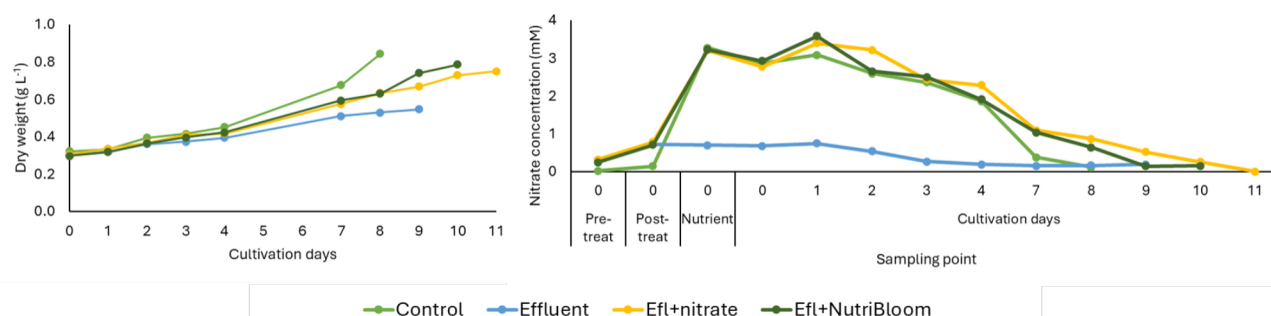


Figure 1. Microalgae culture dry weight and nitrate concentration of *Nannochloropsis oceanica* in an outdoor 100L FP-PBR under four experimental treatments: (Control) seawater supplemented with NutriBloom®; (Effluent) effluent without nutrient supplementation; (Effluent+nitrate) effluent supplemented with nitrate; and (Effluent+NutriBloom) effluent supplemented with NutriBloom®. Nitrate concentrations were determined before chemical treatment (Pre-treat), after chemical treatment (Post-treat), following nutrient supplementation (Nutrient), and throughout cultivation (Days 0–11).

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Results

The initial nitrate concentration in the RAS effluent was 0.29 ± 0.04 mM and increased to 0.74 ± 0.04 mM after chemical treatment and to 3.21 ± 0.01 mM following nitrate supplementation, reaching the target cultivation concentration (Fig. 1B). NutriBloom® supplementation also increased phosphate concentration from 0.21 to 0.31 mM. The Effluent treatment showed the lowest biomass production, increasing from 0.30 to 0.55 g L^{-1} by day 9, with nitrate remaining below 0.80 mM throughout cultivation and reaching 0.19 mM at the endpoint (Fig. 1A,B). Control treatment showed the highest *N. oceanica* dry weight, increasing from 0.32 to 0.84 g L^{-1} by day 8, when nitrate concentration had decreased from 2.86 to 0.12 mM. Effluent + nitrate and Effluent + NutriBloom, showed similar *N. oceanica* growth and nitrate consumption, both higher than the Effluent treatment and slightly lower than the Control, reaching final biomass concentrations of 0.75 and 0.79 g L^{-1} . Nitrate was progressively consumed in all supplemented treatments, reaching 0.12, 0.00 and 0.16 mM at the end of the Control, Effluent + nitrate and Effluent + NutriBloom cultures, respectively.

Discussion

The use of flatfish RAS effluent as a nutrient source for *N. oceanica* cultivation supported its growth, although biomass productivity remained lower than in the supplemented treatments. The low initial nitrate concentration in the Effluent treatment, together with the improved *N. oceanica* growth observed following nitrate addition, indicates that nitrogen availability was a major limiting factor. However, NutriBloom supplementation resulted in higher growth than nitrate supplementation alone, despite comparable initial nitrate concentrations, suggesting that other nutrients also contributed to culture performance. Overall, these results demonstrate the potential of flatfish RAS effluent as a nutrient source for microalgal biomass production. Furthermore, optimising flatfish effluent through nutrient supplementation for *N. oceanica* cultivation can more enhance waste stream bioremediation while recovering its nutrients through the production of valuable microalgae biomass for aquaculture applications.

Acknowledgment

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ITERATIVE PROTOTYPING IN AQUACULTURE RESEARCH AND INNOVATION PROJECTS

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Applied research and innovation (R&I) projects traditionally follow a linear development model. While appropriate for well-defined problems, this approach can be ill suited for applied R&I addressing novel or highly innovative topics. In a number of large, EU-funded aquaculture projects, including PrimeFish, ClimeFish, AquaVitae, and OCCAM, we have tried out an iterative development approach where we deliver a sequence of prototypes of increasing functionality and complexity. This approach provides a structure and a schedule for how to engage users of the project outputs and other interested stakeholders. Initial requirements for the project outputs are established by consulting them at the onset of the project, and normally two prototypes of project outputs are delivered for testing and feedback before the final version is produced at the end. This ensures that users get to see and to some degree use and test the outputs at pre-defined stages of the project, so that feedback can be given and requirements can be revised if needed. This reduces the chances of producing outputs that are not seen as relevant and increases the feeling of ownership by the users who have actively been part of the development process. Having common deadlines across the project for delivering the prototypes also makes stakeholder interaction more predictable and minimises stakeholder fatigue (which is a common problem in projects like this) as all prototypes are available and can be tested at the same time.

Initially, in PrimeFish and earlier seafood projects, we tested the iterative approach on a limited number of outputs that were deemed suitable for prototyping. This worked well, and from ClimeFish and onwards the iterative approach has been the main process model for the entire project, with common prototype delivery deadlines for a range of outputs of different types. This presentation will outline pros and cons of the iterative approach, both in general and in particular for aquaculture R&I projects.

Acknowledgments

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BEYOND THE BUZZ: HOW SCIENCE CREATES STRATEGIC VALUE FOR STAKEHOLDERS AT INDUSTRY TRADE FAIRS

Vidar Onarheim (1) & Peter S. Christensen (2)

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Practical experience, AI and data for stronger trade fair results | Supported by Innovation Fund Denmark

Industry trade fairs represent one of the most critical, yet underutilized, arenas for science communication. While academic conferences excel at peer-to-peer knowledge dissemination, industry trade fairs function as “temporary clusters” [1] where commercial decisions, supplier selections, and technological adoptions are made in real time. However, a significant knowledge gap exists regarding the specific mechanisms through which scientific research translates into measurable value in these high-noise commercial environments. A systematic review of 125 trade fair studies confirms that while trade fairs are recognized as knowledge-intensive environments, the strategic role of the individual researcher—and the facilitating role of the trade fair organizer—remains largely unexplored [2].

This joint presentation brings together two complementary perspectives: the operational experience of a leading Nordic aquaculture trade fair and conference organizer (Aqkva / HavExpo), and the research-based methodology of Expogenius—a platform supported by Innovation Fund Denmark dedicated to transforming trade fairs into measurable value. We ground our analysis in empirical visitor data from HavExpo 2022 - 2026, which reveals that the two dominant visitor objectives are “discovering new technology” and “relationship building”—far surpassing direct purchasing intent. Furthermore, while visitors show massive interest in innovation and aquaculture technology, they rarely identify “research” as a primary interest area. This exposes a critical communication gap: there is immense demand for the knowledge science produces, but researchers remain largely invisible as actors. We address the core thesis: *Science is not merely content to be presented at these fairs; science is a primary driver of strategic value for all core stakeholders, and organizers must actively design for this.*

Drawing on Bathelt’s (2010) “global buzz” theory [3], D’Este and Patel’s (2007) research on university-industry linkages [4], and Nygaard’s (2008) principles of learning-centered education [5], we propose that scientific knowledge creates stakeholder value through contextual translation and pipeline conversion. To empirically test this thesis, we present findings from a three-phase longitudinal study conducted at HavExpo 2026 (Norway, May 2026): (1) pre-fair measurement of visitor objectives and interest areas; (2) post-fair assessment of knowledge outcomes and behavioral intentions; and (3) a six-week follow-up measuring concrete actions taken. All three phases will be completed prior to this presentation, allowing us to present a full empirical pathway from visitor expectation to research-driven impact — the first study of its kind in the Nordic aquaculture sector. The presentation introduces the **Expogenius Strategic Value Framework**, developed through this empirical analysis across multiple Nordic aquaculture trade fairs. This framework demonstrates how science delivers specific outcomes for three distinct groups:

1. **Decision-Makers (Visitors):** Providing evidence-based solutions, risk mitigation, and objective insights that directly support strategic procurement decisions.
2. **Suppliers (Exhibitors):** Offering technological validation, third-party credibility, and pathways to long-term industry contracts.
3. **Platform Organizers:** Generating intellectual authority and the collective legitimacy required to attract top-tier decision-makers and position the fair as a knowledge-intensive platform [6].

Crucially, we argue that trade fair organizers must transition from passive logistics providers to active knowledge curators. To operationalize this, we present a practical “**Tasks First**” methodology — grounded in Nygaard’s (2008) learning-centred design principles [5] — for researchers and organizers alike, detailing actionable strategies for the pre-, during-, and post-fair phases. Key focus areas include: (1) **Stakeholder Targeting:** identifying the right 1,000 people rather than broadcasting to a random million; (2) **Message Distillation:** focusing on the stakeholder’s learning outcome rather than the sender’s dissemination; and (3) **Pipeline Conversion:** transforming the temporary buzz of the fair into long-term knowledge pipelines through structured, data-driven follow-up [7].

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Attendees will leave with concrete, immediately applicable strategies for making their research visible, relevant, and influential. By mastering this arena, researchers can position themselves not just as communicators of science, but as strategic advisors and key influencers in industry innovation networks. The findings from this study are currently influencing the structural design of Global Aqua 2027 (Oslo, Norway) — a new international arena built explicitly to integrate research as a core driver of commercial and technological innovation in aquaculture.

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EFFECT OF TEMPERATURE ON *IN VITRO* PROTEIN HYDROLYSIS OF INSECT BASED DIETS BY THE DIGESTIVE ENZYMES OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

In vitro digestion assays could represent a reproducible and ethical approach to estimating protein bioaccessibility under controlled conditions, allowing rapid and reliable evaluation of novel ingredients and feeds also in the aquaculture sector. Environmental factors such as temperature can significantly influence animal digestive physiology and enzymatic activity, hence *in vitro* hydrolysis outcomes (Wang et al., 2021). Although *in vitro* models should ideally mimic species-specific physiological conditions, including rearing temperature, many studies adopt a standardized temperature (37 °C) to accelerate protein hydrolysis.

Materials and Methods

This study simulated the digestion of *Hermetia illucens* and *Tenebrio molitor* meal based diets, formulated either individually or in combination, as well as their corresponding insect meals, under different temperatures (18 °C, 25 °C, and 37 °C) using digestive enzyme extracts from rainbow trout (*Oncorhynchus mykiss*). Digestions were carried out for 180 minutes, with sampling every 30 minutes. The release of free amino acids over time and the degree of protein hydrolysis were evaluated.

Results

Free amino acid release generally increased with digestion time and was highest at 37 °C, while 18 °C resulted in the lowest hydrolysis. Temperature affected the free amino acid released over time in both insect meals where amino acid release was higher at 37 °C compared to 25 °C and 18 °C. Free amino acid release increased progressively during *in vitro* digestion across all diets, as depicted in figure 1, with temperature significantly affecting both the rate and extent of proteolysis. Hydrolysis was greatest at 37 °C, intermediate at 25 °C, and lowest at 18 °C. The *H.illucens* based diet showed slightly greater amino acid release than the control, particularly at 25 and 37 °C, whereas the *T.molitor* based diet exhibited an earlier saturation of amino acid release and substantially lower hydrolysis at 37 °C. The combined insect meal diet showed a similar pattern to the *T. molitor* based diet. SDS-PAGE analysis confirmed progressive degradation of dietary proteins during digestion, supporting the temperature and diet dependent differences observed in free amino acid release.

Discussion

The results demonstrate that incubation temperature strongly influences protein hydrolysis by rainbow trout intestinal proteases, consistent with poikilothermic nature of *O. mykiss* (Volkoff and Rønnestad 2020). The greater amino acid release observed in both insect meals and diets at 37 °C likely reflects enhanced enzyme substrate interactions and protease activity but may overestimate digestive capacity under physiological conditions. Differences among insect based diets further indicate that protein matrix characteristics and substrate accessibility influence hydrolysis, with the higher chitin content of *H. illucens* potentially limiting enzyme accessibility during early phases of digestion. Overall, 25 °C appears to provide a suitable compromise for *in vitro* digestibility assays using *O. mykiss* intestinal extracts, with hydrolysis patterns closer to those observed at 18 °C while avoiding the potential overestimation associated with 37 °C.

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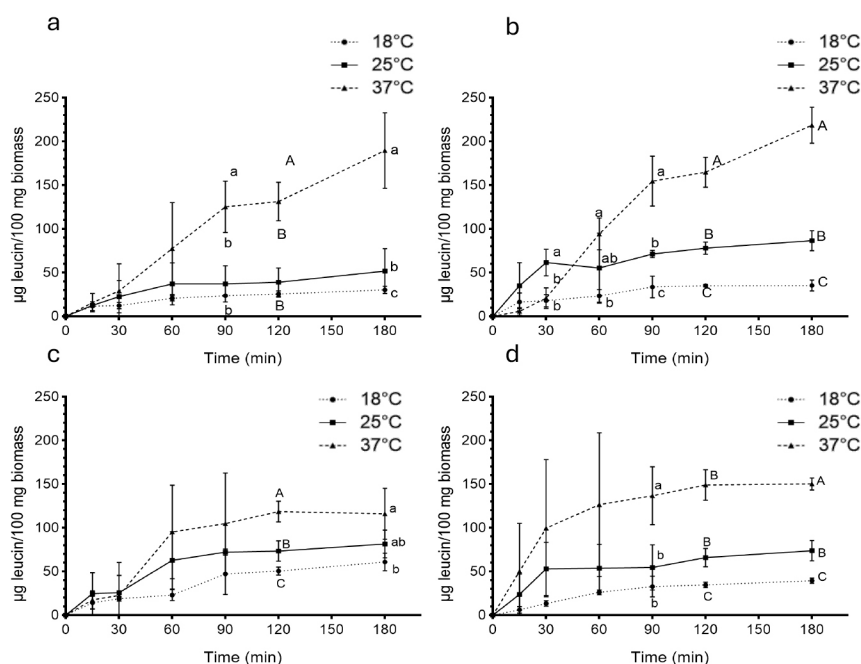


Figure 1. Effect of temperature (18 °C, 25 °C, and 37 °C) on the release of free amino acids (µg leucine/100 mg protein) during the *in vitro* digestion of (a) CTRL, (b) H, (c) T, (d) HT diets by *O. mykiss* intestinal extracts. Data are presented as mean ± standard deviation (SD) (n = 3) for each time point. Different letters indicate significant differences among different temperatures (a, b, c $p < 0.05$; A, B, C $p < 0.001$).

Funding

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PROGRAMMING EFFECTS OF EGG-STAGE TEMPERATURE AND EARLY-LIFE DIETARY LIPID LEVEL ON RAINBOW TROUT PERFORMANCE TO HARVEST

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Introduction

Rainbow trout is Norway's second largest aquaculture species, yet it experiences substantial production losses in both freshwater and seawater phases, comparable to those observed in Atlantic salmon. Healthy development of rainbow trout is established during early embryogenesis, when temperature critically regulates organ formation. Deviations from an optimal temperature in this phase increase deformities and mortality, yet the effects of temperature fluctuations during key developmental windows remain insufficiently understood. Although high dietary lipid levels and selection for rapid growth promote increased fat deposition and altered body composition, the role of egg-stage temperature and whether males and females respond differently to these early-life conditions in terms of muscle development and fish quality, remains largely unknown. This study examines how embryonic temperature and early dietary lipid levels interact to shape growth, metabolism, and physiological outcomes in all female and mixed sex rainbow trout groups.

Material and methods

Fertilized rainbow eggs were incubated at two temperatures (6°C and 10°C) until hatching, after which all fish were reared under the same temperature regime. From first feeding to ~20g body weight, fish were assigned to three diets differing in lipid content (12%, 18%, and 24%) and to fish groups with all female or mixed sex. Growth, proximate body composition, and physiological parameters were monitored from early stages through seawater transfer and up to harvest size. The mitochondrial respiratory capacity in heart was evaluated at 20g and ca 3000g using a Seahorse Analyzer, while gene expression of muscle markers was investigated in muscle at 20g and 100g. Lipid deposition in different fat depots was analyzed by OilredO staining at 5, 20 and 100g.

Results and discussion

Temperature during the egg stage significantly affected growth and metabolic programming of rainbow trout. Prior transfer to seawater when the fish was approximately 100 gram, the all-female group had a higher SGR than mix sex group, and the 10°C group exhibited higher SGR than the 6°C group. There was no significant dietary effect on growth. However, body composition analyses revealed differences in lipid and protein deposition across dietary, temperature, and all-female versus mixed-sex groups, with the high-protein/low-fat diet resulting in higher protein deposition than the high-fat diets, particularly at 20 g. Both temperature, diets and all-female/mix sex affected gene expression of muscle markers in trout at 20 and 100g. The 6°C group showed enhanced mitochondrial oxidative capacity in cardiac tissue at 100 g, suggesting developmental programming effects. Dietary lipid levels had modest effects on early growth in freshwater but influenced performance in a temperature-dependent manner in sea cages. Increased dietary lipid levels enhanced growth in the 10°C group, in particular in the mixed sex group, while the opposite trend was observed in the 6°C group at slaughter. Differences between all-female and mixed-sex groups were also observed, with all-female fish generally exhibiting higher condition factors and lower average weight than the mixed sex group.

Conclusion

Early-life nutritional and temperature conditions influenced long-term growth, fat deposition, and physiological traits differently in mixed sex and only female groups. These findings demonstrate that embryonic temperature and early dietary lipid levels play a critical role in shaping long-term performance in rainbow trout. The results highlight the importance of early-life programming and suggest that optimizing incubation conditions and early feeding strategies can improve growth efficiency and product quality.

Acknowledgment

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EFFECTS OF STOCKING DENSITY ON GROWTH, METABOLIC PROFILE, AND HISTOLOGICAL CONDITION IN JUVENILE PIKEPERCH (*Sander lucioperca*)

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Introduction

Pikeperch (*Sander lucioperca*) is a freshwater species of increasing interest for intensive aquaculture in Europe due to its high market value, rapid growth, and high-quality flesh (Policar et al., 2013; Pourhosein-Sarameh et al., 2024). Production is typically carried out in Recirculating Aquaculture Systems (RAS), which allow for controlled environments and shorter growth periods. However, the high operational and energy costs of RAS necessitate the maximization of production efficiency to ensure economic sustainability (Kučera et al., 2025). Stocking density is a fundamental husbandry factor that directly influences productivity, particularly during the critical juvenile on-growing phase (Kozłowski et al., 2024). While various environmental parameters such as photoperiod and temperature have been studied, specific research on optimal stocking density for pikeperch remains limited and results are often variable (Policar et al., 2019). Therefore, this study aims to evaluate the effects of different stocking densities on juvenile pikeperch reared in a RAS. The focus is placed on monitoring growth performance, blood biochemical parameters and histological morphology of liver, spleen and gills to establish efficient management protocols for this species.

Material and methods

The study was conducted over 93 days using 6,084 juvenile pikeperch randomly distributed into four groups: Control (10 kg.m⁻³), Low Density (LD, 30 kg.m⁻³), Medium Density (MD, 50 kg.m⁻³), and High Density (HD, 70 kg.m⁻³), each in triplicate tanks. Every two weeks the biomass of each tank was calculated and retired to maintain the density. Growth performance (BW, TL, SL, FC, SGR, FCR) and survival rate were evaluated biweekly, alongside a four-point scale assessment of fin condition. At the beginning (n=10) and end (n=9 per group) of the trial, biological samples were collected for multi-organ analysis. Histological examinations of the liver, spleen, and gills were performed using hematoxylin and eosin staining on 5 µm paraffin sections to assess structural changes. All water quality parameters were maintained within optimal ranges for the species throughout the experiment.

Results and discussion

Survival was exceptionally high across all treatments (>99%), indicating that juvenile pikeperch successfully adapted to intensive RAS conditions up to 70 kg.m⁻³. Although high individual size variability was observed, a common trait in this species often linked to social hierarchies (Ljubobratović et al., 2016; Szkudlarek et al., 2007), standard length (SL) significantly increased in the HD group compared to the initial state ($p < 0.05$). Blood biochemistry revealed a significant decrease in cholesterol and amylase, alongside a downward trend in triglycerides, total protein, and albumin as density increased. These findings, combined with the transition from vacuolated to eosinophilic hepatocytes observed histologically, suggest a metabolic shift where stored energy reserves were mobilized to meet the demands of the environment (Pěnka et al., 2026). Histological adaptations in the spleen (congestion and larger MMCs) and gills (mild basal hyperplasia) represent non-pathological responses to rearing conditions. Crucially, the decrease in ALT and AST levels compared to initial values confirms the absence of liver damage (Ostolaza Perez-Cruz et al., 2026). In conclusion, stocking densities up to 70 kg.m⁻³ are viable for intensive pikeperch production, as the observed physiological and histological adjustments remained within a healthy adaptive range, ensuring high survival and growth performance without compromising fish welfare.

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Acknowledgment

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HOW FRAGMENTED EU GOVERNANCE AND LICENSING, ARE LOCKING AQUACULTURE INTO MONOCULTURES

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Introduction

While aquaculture production is expanding globally, EU aquaculture output remains relatively stagnant and continues to cover only a limited share of domestic seafood demand (HUNTINGTON and CAPPELL 2026). At the same time, policy priorities increasingly emphasize diversification, particularly through low-trophic species and more resource-efficient production systems. However, this transition remains slow. We argue that this reflects a “diversification paradox”: while policy ambition promotes diversification and sustainability, existing governance and permitting systems continue to favour established single-species production models (Chan, Cai et al. 2024, Lester, Gentry et al. 2024).

Materials and Methods

This contribution adopts a policy analysis perspective, examining how EU-level frameworks and their national implementation shape investment conditions in aquaculture. The analysis focuses on key regulatory domains affecting site development, environmental compliance, and market access, and explores how their interaction influences the feasibility of diversified production systems.

Results

The analysis reveals a severe structural mismatch: diversified, circular systems face significantly higher compliance burdens than traditional monocultures. A licensing architecture designed primarily for single species and linear outputs forces multi-species systems to undergo multiple parallel approvals. It also consistently treats valorised side-streams as regulatory “orphans” and introduces inconsistent interpretations across different jurisdictions (Alexander, Potts et al. 2015, Camarena-Gómez, Lähteenmäki-Uutela et al. 2022). Empirical data indicates that this administrative complexity is worsening rather than improving, contributing to a 33% decline in new aquaculture licences and uneven processing times across Member States. Ultimately, these fragmented permitting structures increase investment risk and transaction costs, thereby locking the industry into established monoculture models that align more easily with existing regulatory pathways (Dixit and Pindyck 1994).

Discussion

EU aquaculture policy is currently advancing in ambition but failing in deployability, resulting in governance systems' coherence limitations (Huntington 2025). In order to overcome this structural bias, we need to go beyond calls for more innovation and implement “diversification ready” regulations to actively align benefits with circular ends; we need to improve policy coherence and reduce the degree of administrative uncertainty as essential conditions necessary for enabling the development of a more sustainable and resilient European aquaculture.

(Continued on next page)

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CONTROLLING MATURATION AND INCREASING PRODUCTION CAPACITY IN SUBMERGED CAGE FARMING OF ATLANTIC COD *Gadus morhua*

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Light management is a key factor in controlling sexual maturation in farmed Atlantic cod, yet conventional approaches based on continuous artificial illumination are being challenged by emerging production strategies. This session presents results from submerged cage systems, where fish are held at greater depths with naturally reduced light intensity. The findings indicate that it is possible to maintain low levels of maturation without relying on high artificial light exposure, suggesting a more energy-efficient production regime. The presentation will highlight recent data on growth performance, maturation control, and operational considerations when combining deep farming with targeted light management. Ode is currently the only cod farming company in the world operating submerged cage systems and is now observing highly promising results from this approach. The presentation will also introduce a novel solution designed to provide even greater control and increased production capacity.

EFFECTS OF RAW, SPROUTED AND HEAT-TREATED MUNG BEAN (*Vigna radiata*) ON JUVENILE TILAPIA (*Oreochromis niloticus*)

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Introduction

Mung bean (*Vigna radiata*) has recently attracted attention as a plant-based ingredient for Nile tilapia (*Oreochromis niloticus*) feeds, either as seed meal or sprout-derived material (Kubiriza et al., 2018; Soliman et al., 2018; Negara et al., 2026). However, its nutritional value may depend on processing, since raw seeds, sprouts and heat-treated materials can differ in palatability, nutrient availability and metabolic effects. This study evaluated the effects of raw, sprouted and heat-treated mung bean on growth performance, feed utilization, serum biochemistry, condition factor and organosomatic indices of juvenile tilapia.

Materials and Methods

The feeding trial was carried out for 80 days in a closed recirculating aquaculture system with experimental tanks of approximately 80 L working volume. Nile tilapia with an initial body weight of approximately 2.97 g were distributed into three dietary treatments: raw mung bean, heat-treated mung bean and sprouted mung bean. Each treatment had three replicate tanks with 10 fish per tank. The diets were formulated to contain approximately 38% crude protein and 6% lipid. Fish were hand-fed to apparent satiation, and water temperature was maintained at 27.5 ± 1.0 °C. At the end of the experiment, growth performance, feed conversion ratio, serum biochemical parameters, condition factor, viscerosomatic index, hepatosomatic index and spleenosomatic index were determined. Data were analyzed by one-way analysis of variance followed by Tukey's test at $P < 0.05$.

Results

All treatments showed a 100% survival rate. Final body weight was affected by dietary treatment, with the highest value in the sprouted mung bean group (17.48 ± 1.15 g) and the lowest value in the heat-treated mung bean group (13.53 ± 0.76 g). The raw mung bean group showed an intermediate response (17.18 ± 0.75 g). Final body length did not differ significantly among treatments, although the sprouted group showed the highest numerical value. Feed conversion ratio was numerically more favorable in the sprouted mung bean group than in the heat-treated group.

Serum biochemical results showed a different trend from growth performance. Total protein, albumin, triglyceride and alkaline phosphatase were higher in fish fed the heat-treated mung bean diet, but these increases were not reflected in improved growth. Alanine aminotransferase, aspartate aminotransferase, glucose, globulin and albumin/globulin ratio were not significantly affected by diet. Condition factor, viscerosomatic index, hepatosomatic index and spleenosomatic index were also similar among treatments.

Discussion

The present results suggest that sprouting was a more suitable processing method than heat treatment for mung bean inclusion in juvenile tilapia diets. The sprouted mung bean diet supported higher final body weight and numerically better feed utilization, without marked changes in condition factor or organosomatic indices. In contrast, heat-treated mung bean increased some serum protein- and lipid-related indicators, but this response did not provide a growth advantage. Therefore, under the present experimental conditions, sprouted mung bean appeared to be the most promising form, while the serum profile of the heat-treated group likely reflected a processing-related metabolic response rather than improved performance.

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AN OPERATIONALLY EFFICIENT FOURTH-TIER FATTENING MODEL FOR ASIAN SEABASS (*Lates calcarifer*) IN COASTAL FLOATING CAGE SYSTEMS

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Asian seabass (*Lates calcarifer*) farming in India largely follows a three-tier cage culture system, with fish harvested at approximately 1 kg body weight despite strong consumer preference for larger-sized fish (>2.5 kg). This study evaluated a fourth-tier fattening model aimed at bridging this market gap by extending the culture of 1 kg seabass for an additional short-duration grow-out phase in floating cages. Field trials were conducted in the brackishwater creeks of Vembanad Lake, Kerala, using $4\text{ m} \times 4\text{ m} \times 3\text{ m}$ HDPE–GI floating cages. A total of 300 seabass (1.0 ± 0.1 kg) were stocked at a density of 10 kg m^{-3} and reared for 120 days using low-value fish as feed.

The fattening model resulted in a mean final weight of 2.5 ± 0.2 kg, with a daily weight gain of $18 \pm 2\text{ g day}^{-1}$ and a specific growth rate of $0.96\% \text{ day}^{-1}$. Survival was high (96.67%), and size uniformity at harvest was excellent ($\text{CV} = 0.07$). The feed conversion ratio was 5.06, acceptable for trash-fish-based feeding in short-cycle fattening operations. The total biomass harvested from the three cages was 3,160 kg.

Economic analysis demonstrated strong financial viability, with a benefit–cost ratio of 1.68, an internal rate of return of 66%, and a payback period of only 0.43 years. The model significantly reduced biological risk by bypassing early life stages while achieving a premium market size within a short culture period.

The results confirm that fourth-tier seabass fattening is a biologically efficient, economically profitable, and climate-resilient mariculture strategy. The model enables a dual-tier supply chain in which small-scale farmers exit at 1 kg. At the same time, specialized fattening units complete the final grow-out, thereby improving income, meeting market demand, and reducing pressure on wild seabass stocks.

FROM LINEAR WATER USE TO CIRCULAR REUSE: AN EU-COMPLIANT TREATMENT STRATEGY DELIVERING TAP-WATER-EQUIVALENT QUALITY FOR AQUAPONICS

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Introduction

Increasing water scarcity, climate pressure, and competition between agricultural, industrial, and urban water uses are driving renewed interest in wastewater reuse as a sustainable water source for food production systems in Europe. Regulation (EU) 2020/741 on minimum requirements for water reuse establishes a harmonised framework to ensure that reclaimed water is fit for purpose, protects human and animal health, and safeguards the environment through a riskbased, multibarrier approach. Aquaponics systems require stable, highquality water to maintain fish welfare, plant productivity, and food safety. Conventionally, these systems rely on drinking water, which offers welldefined and stable physicochemical and microbiological characteristics. In contrast, reclaimed municipal wastewater presents greater variability and potential risks, as standard urban wastewater treatment processes are not designed to meet aquaculturegrade requirements or to fully control pathogens, nutrients, and contaminants of emerging concern. This work focuses on bridging this gap by evaluating whether appropriately designed, integrated treatment trains can produce reclaimed water of quality comparable to tap water for aquaponic applications.

Materials and methods

An advanced tertiary treatment train developed by Aquasoil was applied to municipal wastewater to produce aquaponicsgrade reclaimed water. The MITOX[®] technology combines highefficiency ozonation with enhanced ozone mass transfer and radical formation, achieving nearinstantaneous contact times (<1 s) and optimised pollutant oxidation [1]. Ozonated effluent was subsequently treated by vacuum ultraviolet (VUV) irradiation at 185 nm to promote hydroxyl radical and hydrated electron generation for organic micropollutant degradation. A downstream bioreactive storage unit (15 m³), composed of granular activated carbon, expanded clay, and lava rock, provided adsorption and biological polishing with a 15 h empty bed contact time at 1 m³ h⁻¹. Aquaponic trials were conducted in two replicated, smallscale closedloop recirculating aquaculture systems (RAS) supplied with reclaimed water and tapwater control. Each system consisted of three 350 L fish tanks stocked with 140 ± 6 tilapia coupled to a DWC unit with 5 m² of lettuce production. Physicochemical and biological parameters were monitored after advanced treatment and during aquaponic operation covering microplastics, metals, organic emerging micropollutants, fecal indicator bacteria, parasites (18S rRNA metabarcoding), and human enteric viruses (SaV, NoV GI/ GII, HAV, HEV, crAssphage, PMMoV). Antibiotic resistance genes, class 1 integron gene (*intI1*) and bacterial community structure were characterised using 16S rRNA gene metabarcoding in water, biofilter rocks, fish and roots.

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Results

After implementation of the multibarrier quaternary water treatment, water quality analyses showed that *Escherichia coli* and enterococci were consistently below the limits of detection (<LOD), PMMoV concentrations below the limit of quantification (<LOQ) and no pathogenic parasite-associated reads detected in reclaimed water-fed aquaponic systems. Metal concentrations remained below regulatory thresholds. Levels of total coliforms, organic emerging micropollutants, and microplastics were higher in the RAS compared to reclaimed wastewater influent. The *intI1* gene used as a proxy for antimicrobial resistance (AMR), was mostly <LOQ or <LOD in water, biofilter, fish, and root samples across both experimental runs. No significant differences in either total bacterial abundance (16S rRNA gene) or *intI1* levels were observed in fish or plant roots between reclaimed wastewater and tap water-fed systems. Overall, aquaponic systems supplied with reclaimed water and tap water exhibited comparable trends across all monitored parameters. The study shows that aligning quaternary advanced treatment processes with aquaponic operational needs and EU reuse regulations enables the transformation of reclaimed wastewater into reliable input water source, supporting system performance and circular water management.

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EARLY-LIFE CONSEQUENCES OF DOMESTICATION IN EURASIAN PERCH: PHYSIOLOGICAL AND MOLECULAR PERSPECTIVES

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Introduction

Fish domestication is a complex, multilevel process that affects phenotypic, physiological, and molecular traits determining performance under aquaculture conditions (Teletchea and Fontaine 2014); however, the timing of its onset, particularly during early ontogeny, remains poorly understood. In Eurasian perch, early development may represent a critical window for the emergence of domestication-related adaptations. Our goal was therefore to determine whether the effects of domestication are already evident during the larval period and to identify the factors underlying larval adaptability to aquaculture conditions. By using domestication as a contrasting phenotype at both the zootechnical and genetic levels, we aimed to identify the key physiological and molecular processes associated with successful adaptation to captive rearing.

Materials and methods

For many years, we have conducted comparative studies on larvae originating from wild (WI) and domesticated (DO) broodstock reared under identical conditions (Palińska-Żarska et al. 2020, 2021). Our research included three wild and three domesticated populations, which were compared using standard zootechnical traits, including hatching rate, mortality, deformity rate, and swim bladder inflation efficiency, as well as temperature and nutritional challenge tests. We also assessed genes and enzymes involved in digestion, stress responses, and immune function. Furthermore, transcriptomic analyses were performed across all six populations to determine whether domestication leaves a consistent molecular signature already during early ontogeny. RNA sequencing was performed at 20 million reads per library using 150-bp paired-end sequencing, allowing us to identify a potential transcriptomic fingerprint of domestication and assess its consistency across independently derived wild and domesticated populations.

Results

No differences were observed between wild and domesticated larvae in deformity rates or swim bladder inflation. However, DO larvae showed higher growth, larger body size, and lower mortality ($p < 0.05$). Domestication effects were evident from the onset of exogenous feeding, with DO larvae exhibiting reduced digestive enzyme activity and altered expression of digestive-related genes (Fig. 1), suggesting reduced digestive plasticity and adaptation to formulated diets. Domestication also affected stress and immune responses. Transcriptomic analysis identified 123 DEGs, mainly associated with oxidative stress, energy production, mitochondrial function, and development.

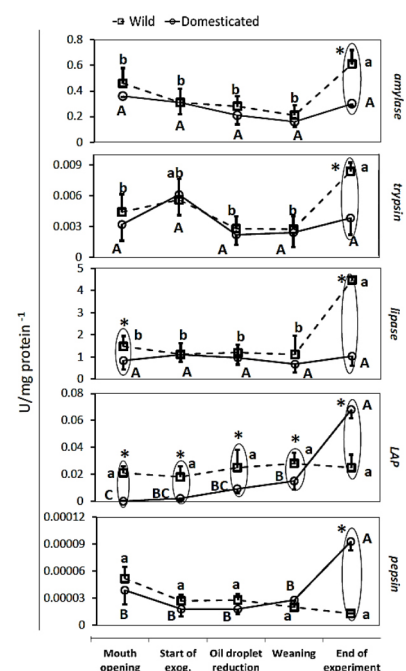


Fig. 1. Expression of genes involved in digestive enzyme production. Values highlighted by ovals indicate statistically significant differences between wild and domesticated larvae.

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Discussion

Our findings demonstrate that domestication is already evident during the larval period and substantially reshapes the Eurasian perch phenotype (Palińska-Żarska et al. 2020, 2021). Farming conditions act as strong modulators, influencing growth, survival, stress responses, and digestive processes. Domesticated larvae appear to acquire distinct stress-coping and digestive strategies, reflecting adaptation to captive conditions rather than simply enhanced performance. We identified candidate molecular markers representing a potential domestication fingerprint, with mitochondrial and metabolic processes emerging as key components of early-life adaptation to aquaculture.

Acknowledgment

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NUTRITIONAL EVALUATION OF METHANOTROPH BACTERIAL MEAL AS A FISHMEAL REPLACEMENT IN JUVENILE RUSSIAN STURGEON (*Acipenser gueldenstaedtii*)

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Introduction

The increasing scarcity and rising cost of fishmeal (FM) challenge the sustainability of aquaculture, particularly for carnivorous species with high dietary protein requirements such as sturgeons. Single-cell proteins (SCPs) produced from microorganisms have gained attention as alternative protein sources due to their high protein content, favorable amino acid profiles, and low environmental footprint. Methanotroph bacterial meal derived from *Methylococcus capsulatus* (Bath) has demonstrated promising results in several teleost species; however, its suitability for sturgeon nutrition remains poorly understood. This study evaluated the feasibility of partially replacing FM with methanotroph bacterial protein meal in diets for juvenile Russian sturgeon (*Acipenser gueldenstaedtii*), with emphasis on growth performance, feed utilization, nutrient digestibility, gut microbiota, and intestinal morphology.

Materials and Methods

Juvenile Russian sturgeon (initial body weight 90 ± 11 g) were fed four isoproteic, isolipidic, and isoenergetic diets for 10 weeks in a recirculating aquaculture system. A control diet contained 30% FM, while three experimental diets replaced FM at 15%, 30%, or 45% with methanotroph bacterial meal. Growth performance and feed utilization were monitored every two weeks. Apparent digestibility coefficients (ADC) of nutrients and energy were determined using yttrium oxide as an inert marker. Gut microbiota structure was assessed by flow-cytometric fingerprinting, and intestinal morphology was evaluated through histological analysis of mid- and hindgut tissues.

Results

Growth performance and feed utilization were maintained in fish fed diets containing up to 30% FM replacement, with no differences compared to the control. In contrast, a 45% replacement level resulted in significantly lower final body weight, total length, specific growth rate, and protein efficiency ratio ($p < 0.05$). Survival and organosomatic indices were unaffected by dietary treatment. Protein and lipid digestibility were reduced at the highest inclusion level, while marked reductions were observed in carbohydrate and phosphorus digestibility ($p < 0.05$). Digestible energy was highest in the 15% and 30% replacement diets and lowest at 45% replacement. No significant differences were detected in gut microbiota metrics among treatments, although numerical trends suggested altered microbial structure at the highest inclusion level. Intestinal histology revealed no pathological alterations, with only an increase in enterocyte height at 45% replacement.

Discussion

The present study reveals a clear inclusion-dependent response to methanotroph bacterial meal in juvenile Russian sturgeon diets. Fishmeal replacement up to 30% supported growth performance and feed efficiency comparable to the control diet, whereas a higher replacement level (45%) resulted in reduced growth and protein utilization. This nonlinear response indicates the presence of a nutritional threshold beyond which the digestive and metabolic capacity of Russian sturgeon is exceeded.

The maintenance of growth and feed utilization at moderate inclusion levels is consistent with findings reported for other carnivorous fish species fed methanotroph bacterial protein, where partial fishmeal replacement supported efficient nutrient utilization when inclusion remained below species-specific tolerance limits. In contrast, growth depression at high inclusion levels has frequently been associated with post-ingestive limitations rather than reduced feed intake. In the present study, unchanged feed intake combined with reduced apparent digestibility of protein, lipid, carbohydrate, and phosphorus at 45% replacement supports this interpretation.

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The pronounced reduction in carbohydrate and phosphorus digestibility at the highest inclusion level likely contributed to the observed decline in digestible energy, providing a mechanistic explanation for the reduced growth despite similar feeding rates. These effects may be linked to the structural complexity of bacterial cell walls and the increased dietary nucleic acid load associated with high SCP inclusion, which can limit enzymatic access to intracellular nutrients and reduce mineral availability. The stability of digestibility coefficients at 15–30% replacement indicates that moderate levels of bacterial protein remain nutritionally accessible to digestive processes in juvenile Russian sturgeon.

Gut microbiota structure and intestinal morphology were largely unaffected by dietary treatment, indicating that growth impairment at high inclusion levels was not associated with intestinal inflammation or microbial dysbiosis. However, numerical shifts in microbial structure at 45% replacement were consistent with reduced nutrient digestibility, suggesting functional modulation of the gut environment rather than pathological alteration. The absence of histological damage further supports the nutritional safety of methanotroph bacterial meal at moderate inclusion levels.

Overall, these findings demonstrate that methanotroph bacterial protein meal is a viable and sustainable alternative protein source for juvenile Russian sturgeon when applied at moderate fishmeal replacement levels, while excessive inclusion compromises nutrient utilization and growth through digestive and energetic constraints

FRESHWATER INTEGRATED MULTI-TROPHIC AQUACULTURE AS A NATURE-BASED SOLUTION FOR NUTRIENT MANAGEMENT AND PROFITABILITY BOOST IN TRADITIONAL CARP FARMING

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Introduction

Freshwater Integrated Multi-Trophic Aquaculture (F-IMTA) represents a promising strategy to improve the environmental sustainability and economic resilience of traditional pond-based aquaculture. By co-culturing complementary trophic groups alongside a primary fed species, F-IMTA systems can intercept excess nutrients, reduce the risk of eutrophication, and diversify farm revenues. In Central and Eastern Europe, common carp (*Cyprinus carpio*) pond farming is a dominant aquaculture model with significant cultural and ecological value, yet it faces growing pressure from environmental regulation, climate-driven water stress, and market competition (Panicz et al. 2022). This work presents the development and demonstration of F-IMTA at the Ińskie Centrum Rybactwa (ICR) farm in Maliniec, northwestern Poland, across two consecutive research frameworks. The F-IMTA concept was first piloted at ICR under the Horizon Europe SmartAqua4Future (SAFE) project, which applies circular-economy approaches to freshwater aquaculture waste streams (Nederlof et al. 2022, Eljasik et al. 2026). The SAFE pilot combined common carp with narrow-clawed crayfish (*Pontastacus leptodactylus*) and watercress (*Nasturtium officinale*) in dedicated ponds separate from the main production. In parallel, the project applied a circular economy approach in which carp sludge and straw bricks served as substrate for oyster mushroom production; the resulting spent mushroom substrate was then used to rear mealworms, with mealworm meal evaluated as a sustainable feed ingredient for carp. In parallel, FISHPLANT, a separate project co-funded by ZUT and ICR through the European Maritime and Fisheries Fund (2022–2023), established new low-input, multitrophic fish-and-plant production infrastructure at the same farm site. Building on these foundations, a full commercial-scale F-IMTA demonstration was implemented during the 2025 production season under the BLUEBOOST project (Sustainable Blue Economy Partnership, Grant No. SBEP2023-725), generating the first high-resolution empirical nutrient mass-balance dataset for carp-based F-IMTA under real operational conditions.

F-IMTA System Design and Deployment

The SAFE project established the initial F-IMTA pilot at ICR using dedicated smaller-scale ponds, demonstrating the feasibility of combining carp with narrow-clawed crayfish and watercress as extractive components. A distinctive circular economy feature of SAFE was the use of carp sludge and straw bricks as substrate for oyster mushroom cultivation; the resulting spent mushroom substrate was subsequently used to rear mealworms (*Tenebrio molitor*), with the produced mealworm meal evaluated as a sustainable feed ingredient for carp, closing the nutrient loop within the farm. The FISHPLANT project (2022–2023), co-funded by ZUT and ICR through the European Maritime and Fisheries Fund, further strengthened the farm's research infrastructure and resilience by establishing dedicated low-input multi-trophic fish and plant production facilities. Under BLUEBOOST, these combined foundations enabled a full commercial-scale F-IMTA demonstration during the 2025 production season. The F-IMTA integrated carp production in an 11 ha pond (pond no. 15) with a floating crayfish cage module (8.07 × 13.90 m, 15 individuals per cage) and a 50 m² watercress production unit positioned in the concrete outflow channel downstream of the pond. Half of the watercress area was covered by a foil tunnel to assess the influence of microclimate on plant growth. Carbon (C), nitrogen (N), and phosphorus (P) flows were quantified using a site-specific mass-balance approach integrating elemental analysis of all system components with a thermal-unit growth model to simulate carp biomass dynamics throughout the full May–November production season.

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Results and Discussion

The ICR demonstration confirmed the technical feasibility of the F-IMTA concept under commercial operating conditions, with all components functioning within the existing farm schedule and infrastructure. The 2025 mass-balance analysis showed that watercress harvested 19.0 kg C, 0.86 kg N, and 0.13 kg P, while crayfish contributed an additional 0.13 kg C, 0.04 kg N, and 0.01 kg P. The overall bioremediation potential of the extractive species relative to feed-derived carp emissions was 0.09% for C, 0.014% for N, and 0.058% for P. Although these values appear low in absolute terms, they reflect the open, flow-through character of semi-intensive pond systems, where the total nutrient pool is highly diluted. The key design principle, i.e. targeting outflow hotspots rather than the entire pond volume, is the critical functional distinction from closed-system IMTA. Additionally, straw bricks placed in the drainage channel sequestered 147.9 kg C, 2.86 kg N, and 0.44 kg P during the season, providing a complementary passive filtration mechanism. These results, derived from longitudinal, site-specific laboratory analyses rather than literature defaults, provide a high-resolution empirical basis for Life Cycle Assessment (LCA) within the BLUEBOOST project and establish a reproducible benchmark for future optimisation of nutrient recovery in temperate freshwater aquaculture.

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Views and opinions expressed are, however, those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them.

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USE OF BLACK SOLDIER FLY (*Hermetia illucens*) AND SPIRULINA *Arthrospira platensis* IN DIETS FOR EUROPEAN SEABASS *Dicentrarchus labrax*

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Introduction

Aquaculture's reliance on fishmeal (FM) as a primary protein source poses significant sustainability and economic challenges due to limited availability, fluctuating prices and pressure on wild pelagic stocks (FAO, 2020). Therefore, it is necessary to identify high-quality alternative source of proteins that maintain growth and nutritional value, especially for carnivorous species like European seabass (*Dicentrarchus labrax*). Cyanobacteria and insects, such as spirulina (SPI, *Arthrospira platensis*) and black soldier fly (BSF, *Hermetia illucens*) have emerged as promising candidates due to their interesting amino acid profiles and rapid production cycles. Nevertheless, the synergistic effects of combining these two distinct alternative proteins in diets remain insufficiently explored. The goal of this study was to replace completely FM with different combinations of Spirulina and BSF to evaluate their impact on the growth performance, health and product quality of European seabass, providing insights into the feasibility of integrated sustainable feed formulations.

Materials and Method

The study comprised two consecutive experiments conducted in recirculating aquaculture systems. In the first experiment, juvenile European seabass (17.33 ± 0.05 g) were fed, in triplicate, seven isoproteic and isolipidic diets for 56 days to evaluate fishmeal (FM) replacement. Diets included a control (CTRL, 15% FM), and four additional diets (MIX1–4) in which FM was fully replaced by BSF and SPI blends at HI:SPI ratios of 80:20, 60:40, 40:60, and 20:80, respectively. Growth performance and somatic indices were recorded. Subsequently, a bacterial challenge was performed by intraperitoneally injecting *Vibrio anguillarum* into fish from the four best-performing groups (CTRL, HI100, MIX1, and MIX2) to assess disease resistance. In the second experiment, these four selected diets were tested over 127 days on larger fish (102.05 ± 0.41 g) until the control group tripled in body weight. In addition to growth performance, product quality of fillets was evaluated by measuring muscle pH, colorimetric parameters (L^* , a^* , b^*), and cumulative water loss (drip, thawing, and cooking loss) to determine the impact of alternative proteins on the final harvestable product.

Results

In the first experiment, the complete replacement of fishmeal with a moderate inclusion of spirulina MIX1 (80/20) and MIX2 (60/40) did not significantly affect growth, feed conversion ratio (FCR) and biosomatic indexes. Following the *Vibrio anguillarum* challenge, a significant linear trend was observed and survival improved in the fish fed the MIX2 diet. Formulations with spirulina content exceeding 60% significantly reduced feed intake, weight gain, and specific growth rate (SGR). Moreover, fish fed SPI100 exhibited a marked increase in FCR and a drastic reduction in hepatosomatic index values compared to the other diet groups.

In the second experiment, results indicated that total or partial replacement of FM led to a significant reduction in growth performance. The CTRL group achieved the highest final body weight and SGR, followed by HI100 and MIX1, while MIX2 showed the lowest growth. FCR and survival was similar across all treatments. Additionally, the fish fed the alternative proteins exhibited a lower condition factors and viscerosomatic index compared to the CTRL group. Regarding product quality, dietary treatments did not significantly affect either muscle pH or the water retention properties of fillets. On the other hand, significant differences were observed in colour parameters; fish from the MIX2 group exhibited significantly higher redness (a^*) in the tail section compared to MIX1, while MIX1 showed significantly lower yellowness (b^*) in the head and mid-sections compared to CTRL.”

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Discussion

The results suggest a moderate SPI inclusion could provide additional protection to the fish when exposed to some pathogens. On the other hand, the significant decline in growth performance and feed intake in diets exceeding 60% SPI suggests that, despite being nutritionally dense, high-level inclusion may impact palatability or metabolic efficiency in European seabass. Nevertheless, a moderate inclusion of *Spirulina* (MIX2) seemed to offer a functional advantage, progressively enhancing survival against *Vibrio anguillarum*. The second experiment suggests that the complete replacement of fishmeal with these two alternative ingredients causes a deterioration of growth parameters when adopted for the entire production cycle. The results indicate that while replacing FM with combinations of BSF and SPI may provide benefits in the short term, additional research is required to understand the limiting factors affecting long term use in European sea bass.

Acknowledgment

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MULTIPLE USE OF SPACE AND OFFSHORE AQUACULTURE: THE MUSICA PROJECT

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Introduction

Although global aquaculture production continues to expand, growth in Europe remains constrained by competing demands for finite areas and resources, stringent regulatory frameworks, and intense competition from non-EU producers (Jansen *et al*, 2016). While land demand for development and other uses compete the needs for space to practice fish-farming, the ocean is still underutilised as a location for protein production, with only 5% of the global protein supply and a mere 2% of the total human calorie intake acquired from these ecosystems (Kaiser & Chambers, 2017). Offshore aquaculture has been identified as a key avenue for growth among European enterprises (Jansen *et al.*, 2016). Its advantages include enhanced water quality from wind- and current-driven oxygenation, lower pathogen risks, more stable temperatures, and reduced competition for space.

However, offshore fish farming demands substantial capital investment, and adverse weather poses ongoing challenges. To mitigate costs, aquaculture systems can be integrated with other offshore infrastructures, diversifying revenue streams and sharing expenses. Multi-Use Platforms (MUPs), operational since the mid-1990s, exemplify this approach; China leads recent efforts, deriving over 20% of its national aquaculture output from them (Yingxin, 2023). Globally, MUPs have encountered bureaucratic hurdles over three decades, including protracted licensing, legal ambiguities in Exclusive Economic Zones, balancing access among activities (e.g., energy extraction and aquaculture), and uncertainties in insurance and liability. The MUSICA project seeks to demonstrate MUPs as viable infrastructures for co-locating energy production, desalination, and aquaculture, thereby sharing offshore space to cut operating and maintenance costs.

Material and Methods

The MUSICA platform will be located offshore the island of Oinousses, near Chios, Greece. It will integrate renewable energy generation from solar, wind, and wave sources with a High-Density Polyethylene (HDPE) aquaculture cage positioned 200 m away. Designed for sea bass or sea bream, the cage features a 2,000 m³ rearing volume, 16 m diameter, and 19.5 mm Dyneema mesh net.

Employing Tension Leg Cage (TLC) technology, the cage submerges automatically via tensioned ropes when waves exceed 3 m. Beyond this threshold, currents deflect it sideways while ropes maintain a horizontal base, ensuring steady positioning in the water column without violent motions. Upon calmer conditions, it returns automatically to its operational state.

Feeding will be relied on an automated Integrated Feeding System (IFS). A 90–110 mm PVC pipe will deliver feed via air blown from the system, with a 2-tonne feed silo and a control cabinet housed in a container on the MUP. Feed bags will be stored there too, requiring manual silo reloading only weekly or every 10 days. Programmable via mobile app or control monitor, the feeder dispenses precise amounts at scheduled intervals, aided by underwater cameras for fish monitoring.

Results

MUSICA's platform is still in the assembly stage, with the submersible cage deployed in April 2026, but not yet stocked with fish. The MUP and the cage are situated far from shipping routes and touristic sites, so there will be no conflicts with other potential users. Dissemination of the whole project, including its innovations and advantages to the local communities, has been conducted. The reduced environmental footprint was communicated, decreasing local population's concerns for aquaculture impact, and increasing their interest in the progress of the project. The costs of the MUP will be maintained low and transparent, to promote replication of the system in small islands or remote areas with small communities. A significant decrease in labour cost will be enabled by the high level of automation.

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The automated submergible cage will reduce stress the stocked fish in case of adverse weather conditions. The water exchange and the oligotrophic conditions present, will enable improved growth rate, disease-free production and improved flesh taste. Water currents will dilute nutrients quickly, preventing eutrophication, one of the main consumers' concerns. The cameras will enable prompt feeding interruption in case of deteriorating water quality affecting the feeding behavior. This will reduce pollution and costs. Renewable energy generated at the MUP will provide all the necessary energy needed for the operations.

The MUP will represent a model system showcasing the advantages of this technology and fostering its adoption in other regions.

Acknowledgment

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COMMERCIAL FRESHWATER INTEGRATED MULTITROPHIC AQUACULTURE SYSTEM FOR THE PRODUCTION OF RAINBOW TROUT, CARP, CATFISH AND DUCKWEED. A SLOVENIAN CASE STUDY

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Introduction

Freshwater aquaculture contributes to about 20% of total aquaculture production in Europe, supporting food security, rural employment, and cultural heritage through species like trout and carp. The sector offers sustainable protein sources amid declining wild fisheries and it holds growth potential for diversified, eco-friendly food production. However, freshwater aquaculture faces challenges such as low product diversity, seasonality-associated limitations in the production, strict environmental rules, bureaucracy, water scarcity, and competition with other sectors for space. These limit expansion and innovation in production methods. Integrated Multi-Trophic Aquaculture (IMTA) enhances farm productivity while promoting circular economy principles and resilience. This polyculture farming system can help to address some of the problems that limit the expansion of the sector.

The present study aims to assess the productivity, the environmental impact and the nutrients balance between different compartments of a commercial freshwater IMTA system for the production of different species located in Slovenia. Moreover, the study investigates the possibility of adding an additional trophic level to the farm by introducing a vertical system for the growth of freshwater and protein-rich plants duckweed. These extractive species should remove dissolved nutrients from the fish effluents while producing an extra source of protein for one of the fish species farmed.

Methodology and expected results

The farm is located near the town of Šmarje pri Jelšah, around 12 km from the Croatian border (site coordinates: 46°14'47.3"N 15°30'40.4"E), at the altitude of 200m above sea level. The water is sourced from an adjacent stream from which it flows to a building containing a flow through system where fingerlings of rainbow trout (*Oncorhynchus mykiss*) are reared. Water effluents from this building are sent to the next building, where a Recirculating Aquaculture System (RAS) for the production of *O. mykiss* is present (4 tanks of 150 m³). The water effluent is then sent to a earthen pond of approximative area of 2166 m², for the farming of carp (*Cyprinus carpio*). The water is pumped from the pond to a third building where a RAS system for the production of African catfish (*Clarias gariepinus*) in anaerobic grow tanks with fish mixing biofloc is present. The solid waste is recycled as biofloc and sent back to the growth tanks where they are eaten by the catfish. The system described will be integrated with another compartment, currently under construction, where the aquatic plants duckweed will be cultured in a vertical system inside a glass house, next to the pond. The duckweed compartment will draw nutrient-rich, warm (20°C) water from the African catfish RAS effluent, and it will use it as a growth medium. The water will then go back to the pond. The high temperature of the water and the artificial lights will guarantee year-round production of duckweed, by-passing seasonal limitations. Duckweed growth will be assessed weekly taking aerial pictures of the growing beds and analysing the coverage using a software for analysis of areas. The plants will be kept at the optimal growing density (between 50 and 80%, Walsh et al., 2021). When the density exceeds this range, biomass will be harvested to reinstate the optimal density. Samples of duckweed biomass will be analysed for total protein, lipids and starch to assess how seasonality levels affect the biomass quality. Once the IMTA system will be integrated with the duckweed vertical farm, the overall nutrients balance of the compartments, fish farm productivity and efficiency and fish quality will be assessed.

Water samples will be collected at the entrance and exit of each compartment (green numbers in the image below) three times per week, at approximately the same time for at least a full year. The samples will be analysed for NO₃, NO₂, NH₄⁺ and PO₄³⁻. Water temperature and pH are monitored constantly. Samples of solid waste will also be analysed to assess the amount of nutrients ending in the sediments. In addition to water parameters, feeding rate, FCR, fish growth and fish biometric parameters will be assessed during the production cycle in all the compartments.

Acknowledgment

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SEAWEED IN ECOSYSTEM ENHANCING AQUACULTURE FOR GROWTH AND SUSTAINABILITY (SEAGROW)

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Introduction

Macro-algae production using regenerative ocean farming (ROF) practices has the potential to support important EU objectives such as zero pollution, circularity, biodiversity restoration and ecosystems protection. To date, production of macroalgae in Europe has largely focused on costly low volume non-scalable methods. A compelling business case for cultivated macroalgae production hinges on demonstrating cultivation systems that scale up seaweed yields, unlock economies of scale, and drive down costs per kilogram.

SEAGROW is a three-year project started in October 2025, funded by EMFAF, aimed at demonstrating how large-scale regenerative ocean farming can work as a viable business model delivering measurable ecosystem benefits.

Project's objectives

SEAGROW is deploying an innovative seaweed farming systems in West Cork (Ireland), delivering ecosystem benefits assessed measuring changes in nutrients, carbon, and biodiversity associated with ROF. SEAGROW aims to drive ROF sector growth through key outputs, including Life Cycle Assessment, ROF Standard Operating Protocols and an ecosystem assessment protocol. An ecosystem credits system for new revenue streams and an eco-labelling system will be designed within the project. A dedicated workpackage will assess ROF compatibility with Marine Protected Areas (MPAs) and policy barriers for seaweed cultivation, not only in the Atlantic, but also in the Mediterranean and Black Sea.

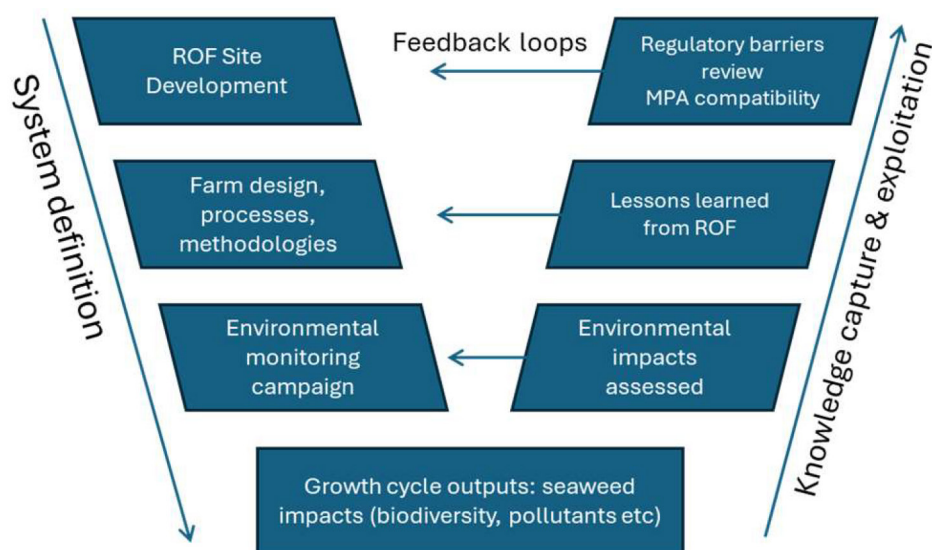


Figure 1. Project implementation methodology represented using the Systems Engineering Vee (V) model (Forsberg and Mooz, 1991).

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Expected outputs and results

The project will generate the technical and environmental evidence needed to de-risk an Irish site for full-scale development by 2031, producing 5,000 tonnes of seaweed annually, with more sites in development using the SEAGROW model. The LCA will identify the production segments responsible for the highest emissions and design operational and logistic modifications to reduce them. A decision-support tool for countries in different regions will be released alongside recommendations for improved regenerative ocean farming and licensing systems. An EU market development strategy will be delivered at the end of the project.

By demonstrating a scalable seaweed farming model with quantified environmental benefits, SEAGROW aims to crowd in private investment and accelerate ROF sector growth in Ireland and across Europe.

As illustrated in Figure 1, the SEAGROW project applies the Systems Engineering Vee model by linking system definition activities on the left side of the Vee, including site development, farm design, environmental monitoring, and growth process methodologies, to corresponding validation and application activities on the right side. Knowledge generated from regulatory reviews, environmental impact assessments, growth cycle outputs, and lessons learned is fed back into the design process through continuous feedback loops, ensuring iterative improvement and adaptive project development.

Acknowledgment

This project is funded by the European Maritime, Fisheries and Aquaculture Fund (EMFAF) under the call EMFAF-2025-PIA-FLAGSHIP. Grant Agreement 101241416.

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AQUAPONIC-DERIVED BACTERIAL INOCULANTS MODULATE LETTUCE (*Lactuca sativa*) GROWTH AND ROOT SYSTEM ARCHITECTURE IN SOILLESS SYSTEMS

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Introduction

Soilless food production systems are increasingly considered as sustainable alternatives to conventional agriculture, which faces mounting pressures from climate change, ecosystem degradation, and disrupted biogeochemical cycles (Richardson et al., 2023). Systems such as hydroponics and aquaponics offer improved resource-use efficiency, reduced reliance on pesticides, and precise control over plant growth conditions, making them well suited for high-yield production in constrained environments (Rodríguez et al., 2022; Gonnella et Renna, 2021). Aquaponics, in particular, integrates aquaculture and plant production through microbial nutrient cycling, reducing dependency on synthetic fertilizers (Lennard et Goddek, 2019). However, nutrient availability in aquaponic systems can be variable and often suboptimal for plant growth due to complex mineralization processes and imbalanced fish-derived inputs (Kasozzi et al., 2019). Biostimulants, especially plant growth-promoting bacteria (PGPB), offer a promising strategy to enhance nutrient use efficiency and plant development. While well studied in soil systems, their application in soilless agriculture remains limited despite the rich and potentially beneficial microbiota present in aquaponic environments (Eck et al., 2019). This study evaluates whether aquaponics-derived bacterial strains can promote lettuce growth and influence root system architecture across different soilless systems and growing cycles, comparing single-strain and consortium inoculations.

Material and Methods

Plant growth-promoting bacteria (PGPB) were isolated from an aquaponic system and characterized *in vitro* for key functional traits, including indole-3-acetic acid production, siderophore production, nutrient solubilization, biofilm production, metabolic activity and antagonism. Selected strains were then evaluated *in vivo* on lettuce grown in controlled, small-scale, soilless systems over the course of two growth cycles. Seedlings were inoculated with either a single strain or a three-strain consortium and cultivated under hydroponic and aquaponic conditions. Aquaponic waters were sourced from trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio carpio*) systems. Plant performance was assessed through measurements of above and below-ground parameters after four weeks of growth. This experimental design allowed the comparison of treatment effects across contrasting systems and the evaluation of the consistency of plant growth responses across trials.

Results

Out of the 31 retrieved bacterial strains, three of them (strains A, H, and T) were selected based on their performance during *in vitro* characterization. Biofilm formation and metabolism depended on strain and medium, with strain T showing the greatest consistency and versatility. Antagonism was limited, though strain A inhibited others in nutrient-rich carp water, reflecting environmental influence. Bacterial inoculation significantly enhanced lettuce growth across soilless systems, with consistent increases in shoot biomass and leaf development relative to non-inoculated controls. These positive effects were observed across water types and trials, although their magnitude varied depending on environmental conditions. Co-inoculation did not consistently outperform single-strain treatments. Root responses were more variable, with treatment effects depending on both water source and trial. Inoculation influenced root system architecture, affecting total root length, proportion of fine roots and specific root length. While root and shoot biomass were positively correlated, root allocation remained unchanged, indicating coordinated growth without consistent shifts in biomass partitioning.

Discussion

Bacterial inoculation improved lettuce growth across contrasting soilless systems, confirming the potential of aquaponic-derived strains as plant growth-promoting bacteria. Aboveground responses were generally robust, with increased shoot biomass and leaf development observed across treatments. However, the magnitude of these effects varied between trials and water sources, highlighting strong context dependency. Co-inoculation did not consistently outperform single-strain application, suggesting that functional redundancy, competition, or environmental filtering may limit consortium benefits. In contrast to shoots, root responses were more variable and revealed substantial plasticity. Changes in root system architecture involved both overall development and shifts in structural traits, indicating adaptive responses to environmental conditions and bacterial treatments. Notably, shoot and root biomass were positively correlated, while root allocation remained independent of shoot growth, suggesting coordinated plant vigor without consistent shifts in biomass partitioning. Together, these results emphasize that while PGPB can enhance plant performance in soilless systems, their effectiveness depends on environmental conditions and inoculation strategies, calling for context-aware optimization of microbial applications without compromising water quality for fish.

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FIRST APPLICATION OF BIOTOXIN AS AN ANTIPARASITIC AGENT AGAINST *Miamiensis avidus*: MECHANISTIC INSIGHTS FROM TRANSCRIPTOME ANALYSIS

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Introduction

Global aquaculture production is steadily increasing, alongside rising reports of pathogen infections, highlighting the urgent need for effective disease control measures. Scuticociliates are opportunistic parasites that infect a wide range of species, including fish, crustaceans, and mollusks. Scuticociliatosis, caused by scuticociliates, has been reported worldwide and causes systemic tissue damage in skeletal muscle, the brain, the liver, the spleen, and the blood. Clinical signs typically include systemic ulceration and hemorrhage, followed by body darkening and abnormal swimming behavior. Formalin and hydrogen peroxide are the most widely used treatments for scuticociliate control. However, both agents were effective only against external parasites and showed limited effect against internal parasites. Furthermore, continuous chemical exposure increases residual drug concentrations in fish tissues and aquatic environments, leading to immune suppression, pathogen resistance, and environmental contamination. Thus, there is an urgent need to develop safe alternative therapeutics to replace conventional chemical treatments. In this study, we evaluated the antiparasitic efficacy of a marine-derived biotoxin against scuticociliates, assessed its safety in host fish, and elucidated its underlying antiparasitic mechanisms through transcriptomic analysis.

Materials and Methods

Miamiensis avidus was aseptically isolated from the brain of infected fish at an aquaculture farm located in Jeju, South Korea. The isolated scuticociliates were cultured using the RTgill-W1 cell line. *M. avidus* was treated with biotoxin at various concentrations and exposure durations, and cellular safety was established through cytotoxicity assessment. Antiparasitic efficacy was evaluated in olive flounder (*Paralichthys olivaceus*) infected with scuticociliates following a 5 hour biotoxin treatment, with observations over 14 days. To assess residual biotoxin levels in fish tissues, muscle samples were collected immediately after treatment, and at 1 and 3 days post treatment. Host safety was further evaluated by monitoring mortality and biochemical parameters over a 28 day period. To elucidate the antiparasitic mechanism of biotoxin, transcriptome analysis was performed. Differentially expressed genes (DEGs) were identified using DESeq2, and gene ontology (GO) enrichment analysis was conducted using clusterProfiler package in R. We identified core co-expression modules through weighted gene co-expression network analysis (WGCNA). By integrating these modules with enriched GO terms, we prioritized candidate genes at the functional intersection to elucidate the key molecular drivers underlying antiparasitic efficacy.

Results

Biotoxin demonstrated 100% antiparasitic efficacy against *M. avidus* at 1,000 µg/L within 4 hours. No cytotoxicity was observed in cells treated with 100 µg/L biotoxin for 5 hours. At this concentration, biotoxin exhibited antiparasitic efficacy against *M. avidus* in infected olive flounder. Fish treated with biotoxin following *M. avidus* infection showed delayed mortality compared to the *M. avidus* only group, and co-treatment of *M. avidus* and biotoxin resulted in a greater than 50% increase in survival rate. No residual toxin was detected in the muscle tissue of biotoxin-treated flounder, and biochemical parameters showed no significant differences compared to the control group over 28 days, indicating no adverse effects on host physiology. Transcriptome analysis identified 6,186 differentially expressed genes (DEGs; $p < 0.05$, $\log_2FC > 1$), of which 3,146 were upregulated, and 3,040 were downregulated. Enrichment analysis revealed upregulation of protein synthesis machinery, lipid metabolism, and ER components, and downregulation of GPCR signaling, ion channel signaling, and antioxidant defenses. WGCNA identified seven modules, of which four showed significant ROS correlation. Hub gene analysis identified ion channel, cytoskeletal, and motility regulators, implicating sensory-motor disruption as the antiparasitic mechanism.

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Discussion

This study shows biotoxin effectively treats scuticociliatosis, killing *M. avidus* rapidly without harming the fish or leaving residues. Infected flounder treated with biotoxin showed improved survival and normal biochemical parameters, confirming its safety. Transcriptome analysis revealed that biotoxin works by suppressing ion channels, GPCR signaling, and cytoskeletal genes. These changes likely disrupt sensory perception and motility. The upregulation of protein synthesis terms may represent a compensatory or stress-related response. These findings elucidate the antiparasitic mechanism of action and suggest that targeting sensory-motor pathways could be a promising strategy for developing novel antiparasitic drugs in aquaculture.

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ADVANCING SOFT-SHELL CRAB PRODUCTION AND EXPORT COMPETITIVENESS IN BANGLADESH: CHALLENGES, OPPORTUNITIES AND FUTURE DIRECTIONS

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Soft-shell mud crab farming in Bangladesh's southwest coast is expanding rapidly due to favorable brackish-water conditions and rising global demand, yet the industry has not fully realized its economic potential. This review synthesizes recent scientific and policy sources and identifies key constraints, including dependence on wild seed, limited hatchery capacity, inconsistent molting, disease and biosecurity gaps, insufficient cold-chain and processing, weak traceability, and lack of international certification, compounded by climate-driven salinity fluctuations. To improve productivity, product quality, and export competitiveness, future efforts should prioritize commercial hatcheries and broodstock management, strengthened farmer training in nutrition and health, digital traceability and HACCP-based quality systems, international certification, climate-resilient farming, and stronger public-private partnerships to accelerate technology transfer.

Introduction

Soft-shell mud crab (*Scylla olivacea* and *S. serrata*) farming has become one of the fastest-growing high-valued aquaculture enterprises in the coastal region of Bangladesh. The southwest coastal districts, particularly Satkhira, Khulna, and Bagerhat, possess favorable brackish water ecosystems that support commercial soft-shell crab production. Increasing global demand, particularly from East and Southeast Asia, together with emerging opportunities in European seafood markets, has created new opportunities for export diversification and livelihood improvement in coastal communities (Bhuiyan et al., 2021; FAO, 2024).

Despite these advantages, Bangladesh has not yet realized the full economic potential of its soft-shell crab industry because of biological, technical, institutional and marketing constraints. This paper reviews the present status of the industry and identifies strategic interventions for improving production efficiency, product quality and export competitiveness.

Current Status, Challenges and Future Directions

This review synthesizes recent scientific publications, government reports, export statistics and industry experiences published during the last decade. Bangladesh possesses several comparative advantages, including favorable climatic conditions, extensive brackish-water resources, skilled farmers and increasing private-sector investment. Soft-shell crab farming offers rapid production cycles, high market value and significant opportunities for employment generation and foreign exchange earnings (Roy et al., 2024).

However, sustainable expansion remains constrained by dependence on wild crab seed, inadequate hatchery technology, inconsistent molting performance, disease risks, limited biosecurity practices, insufficient cold-chain facilities, weak traceability systems, lack of internationally recognized certification, and increasing climate-induced salinity fluctuations in coastal ecosystems (Haque et al., 2025). These challenges reduce productivity and restrict access to premium international seafood markets.

Future development should focus on commercial hatchery establishment, improved broodstock management, farmer training, nutrition and health management, digital traceability, processing infrastructure, HACCP and international certification, climate-resilient farming systems and stronger public-private partnerships. Integrating research institutions, universities, exporters, development partners and government agencies will accelerate technology transfer and improve sector competitiveness.

Bangladesh possesses significant comparative advantages to become a globally recognized supplier of premium soft-shell crab products. Strategic investment in innovation, value-chain upgrading and sustainable aquaculture practices will strengthen export competitiveness while contributing to the Blue Economy, food security and improved livelihoods in coastal communities.

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Conclusion

In conclusion, Bangladesh's soft-shell mud crab industry holds significant potential for economic growth and export diversification. Addressing key challenges—such as seed dependence, hatchery capacity, biosecurity, and climate resilience—through strategic investments in hatchery development, farmer training, traceability, and international certification is crucial. Strengthening public-private partnerships and fostering innovation will enable the industry to realize its full potential, contributing to sustainable development, food security, and improved livelihoods along the southwest coast.

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ECONOMIC SUSTAINABILITY OF HALOPONICS : A CASE STUDY

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Introduction

Haloponics can be defined as aquaponic systems operating with brackish water at salinity levels ranging from 1 to 35 [g/L], while Maraponics indicates systems operating with seawater, e.g. salinity at or above [g/L]. Compared with freshwater aquaponics, these systems can operate in arid coastal areas and are resilient to climate change related water shortage. Furthermore, marine fish species which can be farmed in both haloponics and maraponics systems, such as seabass and seabream, have, in general, a higher market value, compared with those farmed in freshwater aquaponics, e.g. common carp, tilapia. On the other hand, the use of saline water restricts the range of plant species which can be co-produced. The economic sustainability of aquaponic systems is often questioned, due to their high energy demand: this issue is even more relevant for attracting investors in new integrated agrifood systems, such as haloponics and maraponics. In this paper we present the results of a case study, in which two classical methodologies were used for assessing the economic sustainability of coproducing seabream (*Sparus aurata*) and marsh samphire (*Salicornia Europea*), a halophyte tolerant to high salinities and commonly found in Mediterranean coastal areas. This combination seems promising, given the increasing interest in samphire as food and nutraceuticals.

Materials and methods

Seabream and samphire were co-coproduced in a pilot plant located at Ca' Foscari University premises from April 2024 to August 2025. Seabream juveniles were stocked in April 2024, average weight 40 grams, and harvested in August 2025. Samphire seedlings were stocked in April 2024: plants were tagged and repeatedly pruned, until November. Pruned biomass was weighted and added to the harvested one to obtain an estimate of the total biomass production per plant. Seedling were restocked in April 2025: individual plant biomass was estimated on a weekly basis by extracting each plant and relocating it after weighting. Seabream were farmed at a low density, about 10 kg/m³, and weighted individually every month. The plant was equipped with automatic sensors for determining water temperature, salinity, dissolved oxygen and pH downstream the fish tanks and the greenhouse every 15 minutes. Alkalinity, Total Ammonia Nitrogen (TAN), Nitrate and Reactive Phosphorus were monitored on a weekly basis. The data were used to identify and validate the Knowledge Base Model (KBM) component of a Digital Twin prototype of an aquaponic plant. The model formulation and the results are presented in detail in the deliverables available at the BeBlue Italy-Slovenia INTERREG project website: <https://www.ita-slo.eu/it/beblue>. The KBM was used to upscale the production and to design a virtual haloponics plant, partitioned into production lines of the capacity of 2000 tonnes/year of seabream each. The virtual plant was used as a reference model to estimate total capital investments and annual operating costs for the transition to an industrial scale. Revenues were estimated based on a price of €8/kg for sea bream and €10/kg for samphire, based on an analysis of current wholesale market prices in Italy. Based on these data, several economic sustainability indicators were calculated. In the first phase, a cost/benefit analysis was performed by discounting the annual cash flow values for the considered 10-year plant lifetime. The set of indicators included: net present value (NPV), internal rate of return (IRR), and payback period (PBT). In a second phase, a forecasted income statement was developed to determine a company's profit and return on investment (ROI).

Results and discussion

The results are summarized in Table 1, which presents the estimated biomass yields of a 2000 tonne seabream production line, and Table 2, which shows Capital Expenditure (CaPex), Operational Expenditure (OpEx), revenues and the economic indicators. The surface of the Green House, see Table 1, was estimated assuming a Nitrogen Use Efficiency of 70%. Based on the results of the pilot, we assumed that the samphire seedling would take 2 months to reach an average weight of 300 grams, using LED lights, giving five crops per year.

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The results presented in Table 2 suggest that a 10-year long investment project in seabream-samphyre halponics is viable and could produce interesting economic outputs. All indicators are positive: compared with investments in other agrifood systems a PBT of five years is fully acceptable. As halponics is still at an early development stage, there is also large margin for samphyre seedling selection and optimization the energy use, which may lead to further reduction of both CaPex and OpEx. However, the market positioning of both fish and vegetable products is crucial for the absorption of the total production quantity, as well as the selling price: the situation to be assessed is significantly different if the final recipients are, for example, large-scale retail trades of the final consumer.

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EFFECTS OF A NOVEL *Ganoderma lucidum* MYCELIUM - BASED FEED INGREDIENT ON FILLET QUALITY OF EUROPEAN PERCH *Perca fluviatilis*

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Introduction

The development of sustainable aquafeeds requires not only maintaining fish growth and health but also ensuring high product quality for consumers. Fermented agricultural side-streams using fungal mycelium represent a promising alternative to soybean meal (Wan-Mohtar et al., 2021; Kalaiselvan et al., 2025; Yegin, 2025). However, their potential impact on quality and sensory attributes of fish fillets remains largely unexplored. This study investigated whether dietary inclusion of mycelium biomass produced via solid-state fermentation, affects the sensory quality of European perch (*Perca fluviatilis*) fillets.

Materials and Methods

European perch were fed diets in which composite mycelium meal (CMM) at 0% (CMM0), 5% (CMM5), 7.5% (CMM7.5), and 10% (CMM10) over 84 days. This corresponded to a replacement of soybean meal by CMM at 0%, 25%, 37.5%, and 50%, respectively. All diets were formulated to be isonitrogenous, isolipidic, and isoenergetic. After the feeding trial, fish were slaughtered under standardized conditions, and fillets were collected for quality assessment. Instrumental analyses included colour measurements (L^* , a^* , b^*), muscle pH determination at multiple time points (at slaughter, after 7 days, and after frozen storage at 3 and 6 months), and texture profile analysis. Sensory evaluation was performed using an A-not A discrimination test (ISO 8588) to assess potential differences between control and experimental groups. Statistical analyses included McNemar tests for sensory data and generalized linear mixed models to account for repeated measures and panelist variability.

Results and Discussion

Fillet colour measurements showed occasional significant differences between groups. However, these were inconsistent across time points and colour channels, and no clear or systematic effects of dietary treatment were observed.

Muscle pH showed minor but significant differences between groups. At slaughter and after 7 days, pH was higher in CMM5 compared to the control. However, after 3 and 6 months of storage, all groups exhibited significantly lower pH values compared to CMM0.

Texture analysis revealed no significant differences among groups except for elasticity, which was lower in CMM7.5 compared to the other treatments.

Sensory analysis revealed no significant differences between fillets from control and experimental groups across all inclusion levels. McNemar tests showed no detectable discrimination between samples ($p > 0.05$), indicating that panelists could not distinguish fish fed mycelium-based diets from conventionally fed fish. These findings were supported by generalized linear mixed models, which confirmed the absence of significant product effects.

Although minor variations in texture, odour, and flavour were occasionally noted by panelists, these differences were inconsistent and likely attributable to natural variability among individual fish rather than dietary treatment. Variability within the control group itself further reduced the sensitivity of discrimination testing, highlighting a common limitation in sensory studies with biological products.

Conclusion

The inclusion of CMM in European perch diets did not negatively affect sensory quality or key physicochemical fillet properties. Minor differences in colour, pH, and texture were inconsistent and not indicative of systematic effects. These results support CMM use as a sustainable aquafeed ingredient without compromising product quality.

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SHORT-TERM DIETARY TRYPTOPHAN SUPPLEMENTATION MODULATES CORTISOL RESPONSES AND SURVIVAL FOLLOWING ORAL *Yersinia ruckeri* CHALLENGE IN DIP-VACCINATED RAINBOW TROUT

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Introduction

Functional feeds offer a promising strategy to improve fish health and reduce reliance on therapeutic interventions in aquaculture. Among functional nutrients, tryptophan has emerged as an important modulator of stress responses, immune function and mucosal defences. However, its potential to enhance vaccination efficacy remains poorly understood. Vaccination outcomes can be influenced by nutritional status through its effects on mucosal and systemic immune responses. Previous findings suggest that dietary tryptophan can modulate immune–neuroendocrine responses following dip vaccination, although its protective effects against infection remain unconfirmed^[1]. This study aimed to evaluate whether tryptophan supplementation potentiates vaccination against *Yersinia ruckeri* in juvenile rainbow trout (*Oncorhynchus mykiss*) by modulating the neuroendocrine response and improving survival.

Material and methods

Juvenile rainbow trout (216 fish; 16.4 ± 0.95 g) were randomly distributed among three independent RAS. Within each system, fish were assigned in a completely randomized design, to triplicate tanks and fed either a control diet (CTRL) or a CTRL-based diet supplemented with tryptophan at 1.32% of feed, corresponding to a 4.5-fold higher than in the CTRL diet. After three days of feeding, all fish were dip-vaccinated for 30 seconds according to the manufacturer's instructions (MSD Animal Health). Following vaccination, all groups were fed the CTRL diet for 25 days.

At 25 days post-vaccination (450 degree-days), fish from System 1 were sampled (0 hours). Fish from Systems 1 and 2 were then challenged by oral gavage with *Y. ruckeri* strain QSP57.1 (1×10^{11} CFU mL⁻¹). Fish from System 1 were also sampled at 3 and 6 days post-challenge to assess the cortisol response to infection. At each sampling point, blood was collected from 9 fish *per* dietary treatment, corresponding to 3 fish *per* tank. Fish in System 2 were monitored daily for cumulative mortality for 15 days following bacterial challenge. In parallel, fish in System 3 underwent the same oral-gavage procedure but received sterile bacterial culture medium instead to serve as a sham-challenged mortality control.

Results and discussion

Fish fed the tryptophan-supplemented diet for the three initial days showed lower plasma cortisol levels than CTRL-fed fish at 25 days post-vaccination. This suggests that the short-term dietary intervention induced a persistent modulation of the neuroendocrine response, consistent with the homeostatic effects previously reported for tryptophan following dip vaccination^[1].

As expected, plasma cortisol increased after oral challenge, particularly at 3 days post-challenge, confirming activation of the stress response associated with infection and/or gavage. Nevertheless, cortisol remained lower in tryptophan-fed fish than in CTRL-fed fish, suggesting that tryptophan attenuated, rather than suppressed, the neuroendocrine response.

This attenuated cortisol response was accompanied by a higher survival rate in tryptophan-fed fish than in CTRL-fed fish following challenge, reaching 96% and 90%, respectively. Although this difference was not statistically significant, results suggest a possible improvement in disease resilience. Nevertheless, the high overall survival and relatively small difference between treatments limit the strength of this interpretation. Overall, these findings support the potential of short-term dietary tryptophan supplementation to promote a more balanced neuroendocrine response and improve disease resilience in vaccinated rainbow trout following bacterial challenge.

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Conclusion

Short-term dietary tryptophan supplementation before dip vaccination induced a lasting modulation of the neuroendocrine response in juvenile rainbow trout, as indicated by lower plasma cortisol levels both before (25 days post vaccination) and after oral challenge with *Y. ruckeri*. Tryptophan-fed fish also showed a higher survival rate than CTRL-fed fish, although this difference was not statistically significant. These findings suggest that dietary tryptophan may support physiological homeostasis and potentially improve resilience to bacterial infection in vaccinated fish. However, additional analyses of immune, metabolic and gut microbiome responses are required to clarify the mechanisms underlying these effects and to determine whether cortisol modulation translates into a consistent and biologically meaningful improvement in vaccine-mediated protection.

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GROWTH OF PIKEPERCH (*sander lucioperca*) AND EURASIAN PERCH (*perca fluviatilis*) IN MIXED AND SINGLE SPECIES RECIRCULATING AQUACULTURE SYSTEM

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This study evaluated the feasibility of biculture rearing of juvenile pikeperch (*Sander lucioperca*) and Eurasian perch (*Perca fluviatilis*) in a recirculating aquaculture system. The fish were reared for 98 days in monoculture and biculture groups in five stocking ratios (perch proportion expressed as % pikeperch: P0, P30, P50, P70, P100). Groups with higher proportions of perch (P30, P50) were terminated after 28 days due to perch aggression and high pikeperch mortality. Subsequent analyzes focused only on the ethically viable groups (P100, P70, P0). Eurasian perch showed improved performance in biculture (specific growth rate 1.99 % day⁻¹ vs. 1.51 % day⁻¹ in monoculture) with high survival (94.4–96.3 %). On the contrary, pikeperch survival decreased sharply in biculture (61.5 % in P70 vs. 94.7 % in P100), while the specific growth rate remained similar (1.50 % day⁻¹). Elevated glucose and triglyceride levels in bicultured pikeperch indicated chronic stress. Fin erosion remained generally low in both species. Overall, Eurasian perch consistently outcompeted pikeperch, leading to compromised welfare and high mortality in pikeperch. Biculture of these two predatory percids under conditions of an intensive recirculating aquaculture system is impractical.

Materials and methods

The 98-day trial was carried out in 15 cylindrical plastic tanks (380 L each, initial density 3.61 kg m⁻³, 120 fish per tank) connected to an experimental recirculating aquaculture system at the University of South Bohemia, Faculty of Fisheries and Protection of Waters, Vodňany, Czech Republic. The light regime was 12L:12D (7 lux at the water surface). The quality was maintained at 21.9 ± 0.56 °C, dissolved oxygen 104.87 ± 17.35 %, pH 6.99 ± 0.22, ammonia 0.37 ± 0.23 mg L⁻¹, and nitrite 0.51 ± 0.18 mg L⁻¹. The fish (four months old) came from combined pond-intensive production. Five stocking ratios were tested in triplicate using automatic feeders (8-hour intervals) with a daily feed ration of 1.5 % of total biomass (adjusted every 28 days). Growth parameters, organosomatic indices, blood biochemistry, and fin condition (0–3 scale) were evaluated. Data were analysed by one-way/two-way ANOVA or Kruskal-Wallis test followed by Tukey's/Dunn's post-hoc test ($p < 0.05$).

Results

Significant differences ($p < 0.05$) were found in survival and specific growth rate (Table 1). Pikeperch survival was markedly lower in biculture (61.5 % in P70) than in monoculture (94.7 % in P100), while Eurasian perch maintained high survival (94.4–96.3 %) and achieved higher specific growth rate in biculture (1.99 % day⁻¹) than in monoculture (1.51 % day⁻¹). Fin erosion was low and not treatment-dependent. Pikeperch in biculture showed elevated plasma glucose and triglycerides, indicating chronic stress. Fat somatic index was significantly higher in bicultured Eurasian perch.

Discussion

The strong competitive imbalance observed demonstrates that Eurasian perch consistently outcompeted pikeperch for resources, leading to suppressed welfare and high mortality in pikeperch despite comparable growth rates (Amoussou et al. 2022b; Thomas et al. 2022). Elevated stress markers in bicultured pikeperch and improved fat deposition in Eurasian perch confirm behavioural dominance rather than direct physical aggression as the main limiting factor. These results align with previous findings on percid polyculture, where ecological niche overlap prevents successful co-rearing (Pěnka et al. 2024a; Polícar et al. 2019).

Conclusions

Biculture of juvenile pikeperch and Eurasian perch in recirculating aquaculture systems is impractical due to severe competitive imbalance, reduced pikeperch survival, and welfare concerns. Monoculture or carefully selected species combinations with complementary ecological niches are recommended for percid aquaculture.

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LONG-TERM COMPARISON OF GREEN, RED MACROALGAE EXTRACTS AND β -GLUCANS ON THE DISEASE RESISTANCE, GILLS INTEGRITY AND WOUND HEALING 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, wound healing, 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 three different dietary treatments and 6 replicates (tanks of 70 L): Control; β -Glucan 0.1%; Algae MSP 0.3% 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. A 4 x 2 mm wound injury in the skin was created by dermatological punch, 5 per treatment and monitored for 50 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 final body weight (69.8 g vs. 69.9 g vs 73.4 g; $p = 0.017$), increased lysozyme activity (79 vs. 71 vs. 117 U/mL; $p = 0.016$), and were the only group which differed from control for survival 11% vs. 10% vs. 40%, respectively (+400%). Likewise, higher nitric oxide NO production was increased in both, fish fed Algae MSP and β -Glucan (100% vs. 118% vs. 108%, $p < 0.001$). Fish fed Algae MSP showed the lowest gills histopathological score among the challenged groups, indicating a 38% reduction in gill damage, 0.85, 0.71, and 0.53, respectively. Wound area decrease and pigmentation reintegration were improve by 22% and 175% respectively in the Algae MSP group. In conclusion, Algae MSP demonstrated the most consistent overall performance, excelling balanced immune modulation, survival and gills integrity and wound healing. The results highlighted the algae-based solution as a tool for strengthening natural defenses in aquaculture due to its broader benefits in long-term use.

Keywords: Health, Survival, Marine sulfated polysaccharides.

COMPARISON OF COMMERCIAL ZINC ORGANIC SOURCES ON GROWTH, TISSUE DEPOSITION AND BIOAVAILABILITY FOR RAINBOW TROUT

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This study aimed to evaluate the effects of different dietary zinc sources on growth performance, tissue zinc deposition, and intestinal morphology of juvenile rainbow trout (*Oncorhynchus mykiss*) in a 60-day feeding trial. A total of 260 fish, ± 30 g were randomly distributed into 20 tanks of 70 L (13 fish/tank) in a closed RAS. Water temperature was maintained at 14–17 °C and dissolved oxygen above 6.0 mg/L. A trial design of five dietary treatments and four replicates were used and only differing by zinc source: T1 control (inorganic Zn) and three organic chelation technologies T2 (Di & Tri peptides-Zn; Olmix), T3 (Methionine hydroxy analogue-Zn), T4 (Methionine-Zn), and T5 (Glycine-Zn). Total dietary zinc was standardized to 250 mg Zn/kg feed and analytically verified at Eurofins international laboratory (ranging from 248.6–267.4 mg/kg). At day 60, growth performance was recorded and 6 fish per treatment were sampled for blood 1 h post-feeding, gills, skin and liver for Zn quantification, and midgut segment for villi and absorptive area morphometry. Data were analyzed using one-way ANOVA followed by Tukey's test ($p < 0.10$) or Kruskal–Wallis when needed by SigmaPlot 11.0. Growth performance significant differences were observed. Final fish weight were the highest in fish fed T2 followed by T3, T4, T5 and least was T1 ($p = 0.095$) which also reflected higher biomass. Zinc concentrations in plasma, erythrocytes, total blood, and liver remained stable across treatments, indicating no statistically significant effect of zinc source on these tissues and organs. Gut absorptive area (mm^2) were higher +17% in all organic sources compared to control group ($p < 0.001$). Skin zinc deposition showed a clear separation between inorganic control and the organic sources which averaged +21% higher deposition ($p = 0.021$). The most interesting findings were in the Zn quantification in the gills from which differences among the chelation technology were more clearly observed. Zn bioavailability in the gills in fish fed the T2 and T4 were significantly higher by +43% and +37% than control and other treatments meanwhile T3 and T4 did not differ from control group ($p = 0.016$). As a conclusion, the di and tri peptides-based Zn (T2) showed the most consistent response followed by Methionine-based (T4), combining the highest final weight with increased Zn deposition in skin and gills, supporting higher functional bioavailability. These results indicate that Zn deposition in the gills could be useful as sensitive endpoints to differentiate chelation technologies in rainbow trout fed diets standardized for total Zn levels.

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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SMART-IMTA – PRODUCTION OF MACROALGAE, INTEGRATED WITH SOLE AQUACULTURE, MANAGED WITH REAL-TIME MONITORING AND ACTUATION SYSTEMS

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The Farm to Fork Strategy of the European Green Deal has acknowledged the potential of algae to become an important source of alternative low-carbon footprint protein and contribute to improve the sustainability and competitiveness of the aquaculture sector. Nonetheless, the European algae industry is still at an early phase lagging behind the overall increase seen at a global level, mostly driven by Asia. Within this context, INNOAQUA aims to pave the path towards the upcoming sustainable and diversified EU in-land aquaculture industry by leaning on the demonstration and mainstreaming of innovative algae-based foods and solutions, and ecology, circularity and digitalization concepts. To this end, the project addressed main barriers hindering the growth of the sector, starting with the demonstration at a pre-commercial level (TRL6-7) of the operational, technical, and socio-economic robustness of digitally enhanced integrated fish and algae RAS-IMTA cultivation systems text.

The DEMO IMTA system is installed at SEA EIGHT's commercial hatchery in northern Portugal. The area, with over 200m² of macroalgae productive area (mainly for *Ulva* and *Gracilaria*), can handle approximately up to 40m³/h of water from SEA EIGHT's Recirculated Aquaculture System (RAS). The SMART-IMTA includes the development and implementation of two main digital innovations by INESC TEC: (i) a novel Multisensing Module allowing for real time water quality monitoring and; (ii) a Digital Twin Backbone (DTB) that will facilitate the ingestion, transformation and storage of the data flows and make them accessible to other processes.

Preliminary results show an average productivity above 4kg fw/m²/month between April and June. These results alone would represent a production capacity above 800kg fw/m²/month for the entire unit. This data can translate into a removal between 50.4 and 72 kg of Nitrogen per year, on a DW basis. However, these results are likely to be improved as we continue to collect data and, most importantly, as the digital technology starts its management inputs. Ultimately this technology will allow the management of the macroalgae production component without the permanent action of a seaweed expert, contributing significantly towards the implementation of the IMTA concept in other commercial RAS.

MODULATING INTESTINAL INNATE IMMUNITY IN EUROPEAN SEABASS (*Dicentrarchus labrax*) JUVENILES WITH BIOACTIVE PROTEIN HYDROLYSATES

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Introduction

Aquaculture is increasingly challenged to improve sustainability, animal welfare and disease resistance while maintaining high production efficiency. In intensive farming systems, fish are frequently exposed to handling, grading and high stocking densities, which induce stress, impair immune competence and increase disease susceptibility (Azeredo et al., 2022). Consequently, functional feeds supplemented with bioactive compounds have emerged as a promising nutritional strategy to enhance fish health beyond meeting basic nutritional requirements. Among these, protein-derived bioactive peptides have attracted considerable attention due to their antioxidant, antimicrobial and immunomodulatory properties, with the potential to improve intestinal health and innate immune responses (Chen et al., 2025). Therefore, this study aimed to evaluate the effects of a diet fortified with bioactive peptides derived from poultry feather hydrolysate and whey protein hydrolysate on the intestinal innate immune response of juvenile European seabass (*Dicentrarchus labrax*).

Materials and Methods

European seabass (*Dicentrarchus labrax*) juveniles (initial weight: 5.32 ± 0.60 g; total length: 8.36 ± 0.36 cm) were randomly distributed into experimental tanks at an initial stocking density of approximately 1,000 fish per tank. Two dietary treatments, each in duplicate, were evaluated: a commercial aquafeed (CTRL) and an experimental functional diet (FOR) fortified with bioactive peptides derived from poultry feather hydrolysate and whey protein hydrolysate. Fish were hand-fed to apparent satiation three times daily. After 7, 14 and 45 days of feeding, eight fish per tank were sampled. Intestine samples were collected, snap-frozen in liquid nitrogen, and stored at -80 °C until analysis. Intestinal innate immune parameters, including lysozyme, total peroxidase, and bactericidal activities, and nitric oxide levels, were determined. Data were analysed using a generalized linear mixed model (GLMM) in Jamovi (v2.7), with diet, sampling time and their interaction as fixed effects. Owing to the non-normal distribution and positive skewness of the response variables, a Gamma distribution with a log-link function was applied.

Results

Intestinal lysozyme activity showed distinct temporal patterns between dietary treatments, with higher levels observed in seabass fed the FOR diet after 14 days of feeding compared with days 7 and 45. In contrast, lysozyme activity in fish fed CTRL was lower after 45 days than after 7 and 14 days of feeding. Total peroxidase activity was overall higher in seabass fed CTRL than in those fed the FOR diet. Bactericidal activity also showed different temporal responses between dietary treatments, decreasing from day 7 to day 14 in fish fed the FOR diet, with lower values than CTRL at day 14, while in the CTRL group, activity further decreased from day 14 to day 45. Nitric oxide levels increased over the feeding trial, with no differences observed between dietary treatments.

Discussion

The FOR dietary treatment fortified with bioactive peptides from poultry feather and whey protein hydrolysates influenced the intestinal innate immune response of juvenile European seabass, particularly through changes in lysozyme and bactericidal activities over time. Rather than increasing all immune parameters, the diet appeared to modify the pattern of the immune response throughout the feeding trial. The lower overall peroxidase activity observed in the FOR group, together with the absence of dietary effects on nitric oxide levels, suggests that the functional diet did not induce an excessive inflammatory response. Bioactive peptides have been associated with immunomodulatory and antioxidant properties, while poultry feather hydrolysates have also been shown to be a sustainable ingredient for European seabass diets without compromising fish health or innate immune status (Campos et al., 2017). Overall, these findings suggest that dietary supplementation with bioactive protein hydrolysates may represent a promising nutritional strategy for modulating intestinal innate immunity in juvenile European seabass.

(Continued on next page)

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GENETIC VARIABILITY AND PARENTAGE ASSIGNMENT IN WILD AND HATCHERY PRODUCED *Ostrea edulis* BROODSTOCK FROM THE MAR MENOR

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Introduction

The restoration of the flat oyster *Ostrea edulis* in the Mar Menor is being advanced through the Mar Menor Oyster Initiative (MMOI), which combines ecological restoration, aquaculture innovation and genetic monitoring. Building on the successful reproduction of the few remaining wild oysters achieved in RemediOS-1 project, the current phase evaluates the genetic variability of wild broodstock and hatchery-produced first-generation (F1) individuals to support the production of genetically diverse seed for restoration pilots. Understanding whether hatchery practices maintain the genetic diversity present in the natural population is essential for ensuring the long-term viability of restoration efforts and for guiding improved larval production methodologies.

Methods

Three broodstock groups (wild, F1, and mixed F1+wild; 14 individuals each) were conditioned at RemediOS hatchery at Centro Oceanográfico de Murcia (COMU) under identical hatchery protocols, and larval releases were monitored individually. Tissue samples from broodstock, larvae and post larvae were preserved in ethanol and processed for DNA extraction using the EZNA Mollusc & Insect DNA Kit. Nineteen microsatellite loci were screened (Lallias et al. 2009), and ten highly informative markers were selected for multiplex PCR. Multiplex pools were optimized with Primer Pooler (Brown et al. 2019). Genotyping was performed by capillary electrophoresis, and genetic diversity parameters were calculated across loci. Parentage assignment was conducted using CERVUS, evaluating compatibility through locus comparisons, mismatch counts, LOD scores and Delta statistics to determine the strength of parental matches.

Results and Discussion

Wild broodstock showed larger size and higher larval output than F1 individuals. Across groups, 6–9 larval releases were obtained, with wild oysters producing the highest total number of larvae. Larval survival was greatest in the wild group and lowest in the mixed group.

The microsatellite panel displayed high polymorphism (mean 19 alleles per locus; H_{Exp} = 0.8994; PIC = 0.8845), confirming its suitability for parentage analyses. Most loci conformed to Hardy–Weinberg equilibrium, except Oed273. Some loci (e.g., Oed174) showed evidence of null alleles, consistent with previous reports in bivalves.

Parentage assignment revealed 15 robust parental matches (48% of offspring), 10 compatible but inconclusive cases, and 6 incompatible assignments. Wild broodstock contributed disproportionately to larval production, and recurrent parental candidates were detected within each group, indicating structured genetic contributions.

Acknowledgments

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UNMASKING THE CULPRIT: IDENTIFICATION OF NON-NATIVE POLYCLAD FLATWORM *Postenterogonia orbicularis* PREYING ON MUSSELS *Mytilus galloprovincialis* FARMED IN THE EASTERN ADRIATIC SEA

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Introduction

Bivalve farming in the eastern Adriatic has faced numerous challenges that affect its productivity. Since the 2020s, an unprecedented mortality of farmed mussels has occasionally been observed in the Northern Adriatic region (Slovenia and Croatia) and in the Bay of Boka Kotorska along the Southern Adriatic coast of Montenegro. The damage to crops has anecdotally been attributed to an extraordinarily high incidence of flatworms heavily infesting mussel-growing structures and frequently found inside the empty shell cavities of dead mussels. Their identity has provisionally been attributed to the autochthonous flatworm species *Stylochus mediterraneus* [1,2]. Similarly, intensive predatory activity by flatworms has previously been reported along the Tunisian [3], Italian [4], and Atlantic coasts [5,6]. Since their initial appearance in the eastern Adriatic, year-to-year variability in the occurrence of predatory flatworms and the extent of mussel harvest losses has been observed. In the present study, a preliminary survey was conducted in summer 2024 along the eastern Adriatic coast to establish the current status of the flatworm invasion and to determine the exact identity of this species.

Materials and methods

The study was conducted at mussel aquaculture farms across three eastern Adriatic regions: the Northern Adriatic (Slovenia: Bay of Piran; Croatia: Bay of Soline, Lim, and Raša), the Central Adriatic (Croatia: Kobli and Bistrina in the Bay of Mali Ston), and the Southern Adriatic (Montenegro: Bay of Boka Kotorska). With the exception of the Bay of Mali Ston, mussel losses above baseline levels were observed at all sampling sites, ranging from moderate to severe (up to 100%). Flatworm specimens were collected from mussel socks and stored for subsequent analyses. Identification was performed through phylogenetic analyses using multiple markers: nuclear *28S* and mitochondrial *COI* and *16S*, based on maximum likelihood and Bayesian inference.

Results and Discussion

All specimens found at the Bay of Piran, Bay of Soline, Bay of Lim, Bay of Raša (Northern Adriatic), and Bay of Boka Kotorska (Southern Adriatic) correspond to the polyclad flatworm *Postenterogonia orbicularis*, a species first identified in the sea around Shikoku Island (Japan) and recently detected in the Bay of Biscay (Spain) [6]. The main features of the flatworms collected at these sites were their high density, large size, persistent infestation, and secretion of sticky mucus. Flatworms with notably different phenotypes collected in the Mali Ston Bay area that were not associated with unusual mortalities correspond to the cosmopolitan species *Discocelis* sp. and *Echinoplana cellerima*.

Given its Pacific origin, the flatworm *P. orbicularis* can be classified as a recent introduction to the Adriatic Sea. This species belongs to a group of polyclad flatworms well known for their regeneration capabilities, but its lifestyle, reproductive cycle, and fecundity in the Adriatic Sea have not yet been investigated. According to local farmers, following mussel infestation, *P. orbicularis* spreads rapidly across growing structures, but the environmental conditions favorable to its occurrence and any preference for specific Adriatic regions are currently unknown. Although the Bay of Mali Ston (central Adriatic) appears less threatened, the risk of further invasion into areas currently free of *P. orbicularis* is substantial.

In its native Pacific range, *P. orbicularis* is considered a predator of concern for aquaculture, with no available mitigation or realistic treatment options [7]. Taken together, the present findings and previous records clearly indicate an urgent need to raise awareness among both farmers and policymakers about the potential for further spread of *P. orbicularis* flatworms and the devastating impact their invasion could have on Adriatic bivalve aquaculture.

(Continued on next page)

Conclusions

This study demonstrates an urgent need for monitoring at mussel farms and targeted research on predatory flatworms to develop effective management strategies. In particular, it is essential for mussel farmers to conduct routine checks as a primary line of defense for detecting anomalous events and the introduction of novel species into aquaculture. Stakeholders should maintain comprehensive translocation records and consult biosecurity experts to mitigate the risk of introducing nonindigenous species.

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SELECTIVE BREEDING POTENTIAL FOR CLIMATE RESILIENCE IN MANILA CLAM: EVIDENCE FROM HEATWAVE AND PATHOGEN CHALLENGE EXPERIMENTS

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⁵ Boosting the resilience of European shellfish production against climate change-related challenges through genetic selection – ShellFishBoost. Grant agreement: GA: SBEP23_00051

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Background

Bivalve molluscs aquaculture has steadily increased over the past decades as a source of healthy food rich in omega-3 and omega-6 fatty acids, with a relatively low carbon footprint. However, like other sessile species, molluscs cannot escape unfavourable environmental conditions and are therefore particularly vulnerable to climate change (CC)-induced stressors such as marine heatwaves (MHWs) and pathogen outbreaks. These events can cause massive mortality, as observed during the 2003 MHW and 2024 (summer period), which led to widespread losses of benthic invertebrates across the Northwestern Mediterranean.

Within this context, selective breeding represents a promising strategy to produce mollusc seed with enhanced tolerance to CC-related stressors. This potential is largely driven by the high within-species genetic diversity of molluscs, which remains only partially exploited.

Methods

To test this potential, within the EU-funded project ShellFishBoost, we experimentally assessed tolerance to key CC-related stressors in the Manila clam, *Ruditapes philippinarum*. We produced an F1 population from controlled crosses involving 20 dams and 20 sires, and exposed juveniles to: *i*) a simulated MHW in the laboratory lasting 21 days, with temperatures oscillating between 31 and 35 °C to mimic natural daily cycles; and *ii*) a natural infection challenge by seeding individuals in the Venice lagoon and monitoring infection by the endemic protozoan parasite *Perkinsus olsenii*.

For the MHW experiment, approximately 1,000 individuals were randomly distributed across 10 aquaria. Tissue samples were collected twice daily from dead or moribund (i.e. susceptible) individuals. After 21 days, water temperature was reduced to 25 °C and mortality was monitored for an additional 8 days. Surviving individuals were classified as resistant and sampled accordingly. Overall mortality reached 55% by the end of the experiment.

For the infection experiment, approximately 10,000 individuals were deployed in the Venice lagoon and *P. olsenii* prevalence was monitored monthly. When prevalence reached ~50%, 1,000 individuals were randomly sampled and preserved in ethanol. Gill tissue was used to quantify infection intensity.

Results

Samples from both experiments were genotyped using a newly developed multi-species Axiom SNP array (~49K SNPs for Manila clam). Of the 1,920 genotyped individuals, 1,905 (>99%) passed dish quality control and call rate thresholds, with an average call rate of 98.5%. A total of 35,284 SNPs (71.4%) were classified as poly-high-resolution and used to reconstruct pedigree and family structure. Combined pedigree and phenotypic data (i.e. MHW survival as a binary trait; *Perkinsus* load as a continuous trait) were used to estimate heritability of “MHW tolerance” and “*Perkinsus* resistance.” Analyses are ongoing, with the goal to assess the feasibility of selective breeding programs to enhance resilience to these stressors in Manila clams.

MULTI-USE IN THE NORTH SEA-BALTIC SEA AREA: EXPERIENCES WITH LOW TROPHIC AQUACULTURE IN OFFSHORE WIND FARMS

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Introduction

The world is facing several crises including climate change, pollution/eutrophication, biodiversity loss and a growing risk of food crisis. Low Trophic Aquaculture (LTA) is seen as a potential solution for multiple crises for two primary reasons; 1) LTA produces healthy food with a very low or negative CO₂ footprint, 2) LTA can reduce eutrophication through nutrient removal and create local biodiversity hot spots by attracting epifaunal and mobile species. LTA production is concentrated in the coastal zone due to practical, economic and environmental reasons. As coastal areas are already occupied by many other purposes in densely populated areas such as Europe, the expansion of LTA sectors is challenging, which limits the development of an otherwise sustainable food production. Concurrently, sustainable energy production at sea is expanding rapidly and occupies increasingly larger areas offshore. It is thus obvious to investigate how to co-locate several marine activities e.g., offshore wind and LTA production to solve several problems at the same time and create multi-use spaces. As part of the EU HORIZON project ULTFARMS, we have studied if LTA production of blue mussels (*Mytilus edulis*) and the seaweed species sugar kelp (*Saccharina latissima*) and dulse (*Palmaria palmata*) is biologically and technically possible in two offshore wind farms in the Kattegat, Denmark.

Materials and methods

In Anholt Offshore Wind Farm (OWF) three longlines were established to grow mussels and seaweed in two seasons with different configuration of the lines in different seasons. At Samsø OWF two tube+net system was established to grow mussels in two seasons. At both locations, marine data loggers were deployed to continuously monitor environmental parameters including temperature, salinity, oxygen, light, Chlorophyll-a, and current velocity. Wind data was retrieved from the farm operators. Seaweed lines were seeded in the laboratory and deployed on site. For mussels, spat collectors (Anholt OWF) or nets (Samsø) were deployed in June. At intervals, and when weather windows allowed, samples were taken from the lines and data loggers were cleaned for fouling and data offloaded.

Results

Physical forcing of ocean currents and wind in both locations were strong. In particular Anholt OWF was challenged by environmental conditions leaving few and narrow operational weather windows due to N-NE currents with mean speeds of 1.36 m/s and many consecutive days of wind speeds above 10 m/s from W-SW. In general, mussel spatfall and growth was successful at both locations, which was corroborated by relatively high chlorophyll-a concentrations in the water. In contrast, growth of both seaweed species was low, likely due to prolonged exposure to low and variable salinity and reduced light availability, as cultivation depths were frequently located within or above a stable halocline/pycnocline despite being placed relatively deep (5 m) to avoid physical forcing.

Discussion

The concept of multi-use of marine space is meaningful in view of the political ambition to minimize Europe's dependency on fossil fuels and hence increase offshore wind energy. Here we show that LTA production of species of mussels and seaweeds is biologically possible to some degree in Kattegat, Denmark - an area with existing and planned offshore wind expansions. Compared to more sheltered coastal zones, operating offshore is, however, generally associated with fewer operational weather windows and higher costs. As a result, LTA production is at present only successful in a few locations in Europe. Next steps for a potential broader implementation of multi-use is discussed.

Acknowledgment

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GROWTH AND CONDITION INDEX OF *Mytilus galloprovincialis* IN THE KRKA RIVER ESTUARY

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Introduction

The Mediterranean mussel *Mytilus galloprovincialis* is one of the most economically important bivalve species in Mediterranean aquaculture, with the eastern Adriatic Sea representing a key production area. Growth performance and condition index are widely used indicators of mussel physiological status and cultivation success, reflecting the influence of environmental factors such as temperature, salinity, and food availability. Although these parameters have been investigated throughout the Adriatic Sea, strong local environmental variability highlights the need for site-specific studies. Therefore, this study evaluated the growth dynamics and seasonal variation in the condition index of *M. galloprovincialis* cultivated under traditional farming conditions in the Krka River estuary, one of Croatia's main mussel farming areas.

Materials and Methods

Study was conducted from August 2022 to July 2023 at a commercial mussel farm in the Krka River estuary (eastern Adriatic Sea, Croatia). Growth performance of mussels was monitored using an individual tagging approach. A total of 500 healthy mussels were selected, measured, individually tagged, reseeded onto traditional nylon mesh cultivation nets, and monitored at three-month intervals. Condition index was evaluated monthly using 30 mussels (6–7 cm shell length) collected from the same farming site. Following laboratory processing, dry tissue weight and dry shell weight were determined, and CI was calculated as: $CI = (\text{dry tissue weight} / \text{dry shell weight}) \times 100$.

Results and Discussion

During the study period, seawater temperature ranged from 14.9 to 22.9 °C, with a mean value of 19.2 °C. Growth patterns differed significantly between size categories, with smaller mussels exhibiting the highest growth rates. Small individuals increased their shell length by approximately 2 cm within three months, while market size (~6 cm) was reached within one year under traditional longline cultivation conditions. The observed growth performance indicates favourable environmental conditions for mussel production in the Krka River estuary and compares positively with growth rates reported for other Adriatic and Mediterranean farming areas, where commercial size is generally reached within 12–24 months. Condition index (CI) showed significant seasonal variation throughout the study period, with mean values ranging from 7.31 to 12.42 and an overall mean of 9.52. The highest CI values were recorded in October–November 2022 and March–April 2023, indicating periods of optimal physiological condition and potentially favourable harvesting periods. The lowest CI values observed in January 2023 likely corresponded to reproductive investment and reduced environmental food availability.

Overall, the results demonstrate that growth performance and physiological condition of *Mytilus galloprovincialis* in the Krka River estuary are strongly influenced by seasonal environmental variability. Continuous monitoring of biological responses and environmental conditions provides valuable information for optimizing cultivation strategies, harvest timing, and sustainable management of mussel production in Adriatic aquaculture systems.

Acknowledgment

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FISHIMPACT DIGITAL TOOLKIT: SUPPORTING SUSTAINABILITY TRANSITION AND COMPETITIVENESS OF FISHERIES AND AQUACULTURE SMEs IN THE ADRIATIC-IONIAN REGION

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The fisheries and aquaculture sectors play an important role in providing nutritious food, supporting employment and contributing to the development of the blue economy. However, small and medium-sized enterprises (SMEs) in the aquatic food sector face increasing challenges related to sustainability requirements, transparent communication with consumers and the adoption of effective environmental, social and governance (ESG) practices. In the Adriatic-Ionian region, there is a need for practical and user-friendly solutions that enable SMEs to assess their sustainability performance, identify improvement opportunities and communicate the added value of their products.

The FISHIMPACT project addresses these challenges through the development of a digital, multilingual toolkit designed to support sustainable development and competitiveness of fisheries and aquaculture SMEs. The toolkit integrates three complementary tools: (1) the Sustainability Self-evaluation Tool, (2) the FISHIMPACT QR Labeling Platform, and (3) the Sustainability Good Practices Searchable Tool.

The Sustainability Self-evaluation Tool enables SMEs to evaluate their performance through qualitative and quantitative indicators covering environmental, social, economic and governance aspects, including resource efficiency, working conditions, health and safety, circularity and socio-economic impacts. The tool provides objective scoring, graphical and tabular outputs, and allows companies to periodically repeat assessments and monitor progress over time. The results obtained through the self-evaluation process are directly connected with the Sustainability Good Practices Searchable Tool, supporting the identification of suitable improvement actions.

The Sustainability Good Practices Searchable Tool supports the transition from assessment to action by providing access to sustainability solutions collected from across the Adriatic-Ionian region. Identified technological and managerial good practices are structured in a standardized format, covering environmental, social, economic and health-related sustainability aspects.

The FISHIMPACT QR Labeling Platform enhances communication between producers and consumers by enabling companies to generate QR codes linked to product information, including nutritional characteristics and sustainability-related messages. This approach supports transparency, improves consumer awareness and helps SMEs develop more effective marketing strategies based on the nutritional and sustainability value of aquatic products.

FISHIMPACT provides an integrated digital approach connecting sustainability assessment, improvement pathways and consumer communication. The developed solutions aim to support SMEs in adopting sustainable practices, strengthening market competitiveness and contributing to a more transparent and resilient aquatic food sector in the Adriatic-Ionian region.

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ENERGY UTILIZATION OF DIETARY MACRONUTRIENTS IN GRASS CARP (*Ctenopharyngodon idella*) AND AFRICAN CATFISH (*Clarias gariepinus*)

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Introduction

Sustainable aquaculture requires a balance between what benefits the environment and what benefits the farmed fish. The over- or underuse of macronutrients in fish feeds occurs when the utilization efficiency of fish for the nutrients in question is unknown. In fish nutrition, energy evaluation systems have been established to evaluate the contribution of digested nutrients to the retained energy of fish. One of the energy evaluation systems is the Net Energy (NE) (Blok et al., 2015; Noblet et al., 1994; van Milgen et al., 2001). In the NE approach, feed is split into its composed macronutrients – protein, fat, and carbohydrates. The digested amount of those macronutrients and the retained energy in the fish body are biochemically quantified and subsequently described using mathematical models to characterize their relationship (Groot et al., 2021; Phan, 2022; Schrama et al., 2018). Although previous studies using the NE approach have covered a diverse range of fish species, from carnivores to omnivores, a herbivore remains unstudied. Grass carp (*Ctenopharyngodon Idella*) is a low-trophic fish that possesses a very herbivorous digestive system with a long intestine and no stomach (Zhao et al., 2020). As grass carp has been the most produced finfish in the world for many years (FAO, 2020, 2022), higher productivity and lower production cost are always of interest. Application of the NE approach can clarify how grass carp utilize energy from digested protein, fat, and carbohydrates, supporting balanced macronutrient use to achieve optimal retained energy that is subsequently used for growth. African catfish (*Clarias gariepinus*), a carnivorous species with feeding habits different from grass carp, was included in this study to compare how efficiently both species gain energy from each macronutrient when fed the same diets.

Materials and Methods

The fish were fed with diets contrasting in the content of macronutrients at high and low feeding levels. Feces were collected and analyzed to determine the nutrient digestibility, and fish bodies were combusted in the lab to determine their gross energy (ISO 9831, 1998). A multiple linear regression equation was built in which digestible nutrient intakes are predictor variables and retained energy is the response variable.

Results

African catfish and grass carp showed the same rate of digestibility for protein, fat, carbohydrates, starch, and energy, implying that the trophic level did not influence the nutrient digestibility of one species. The Net energy equation of grass carp was estimated as $NE = 11.7 \times dCP + 45.8 \times dFat - 5.2 \times dFat^2 + 11.5 \times dCarbs$, and that of African catfish was fitted as $NE = 16.9 \times dCP + 40.0 \times dFat + 5.1 \times dCarbs$. African catfish could utilize 100% the digested fat for the retained energy, while grass carp showed a significant ($p = 0.05$) quadratic decline in the retained energy at high levels of digested fat. On the other hand, grass carp showed a significantly ($p < 0.05$) higher energy utilization efficiency for carbohydrates compared to African catfish. Based on these results, cautious inclusion of dietary fat is recommended for grass carp, whereas carbohydrate inclusion should be carefully controlled in the diet of African catfish.

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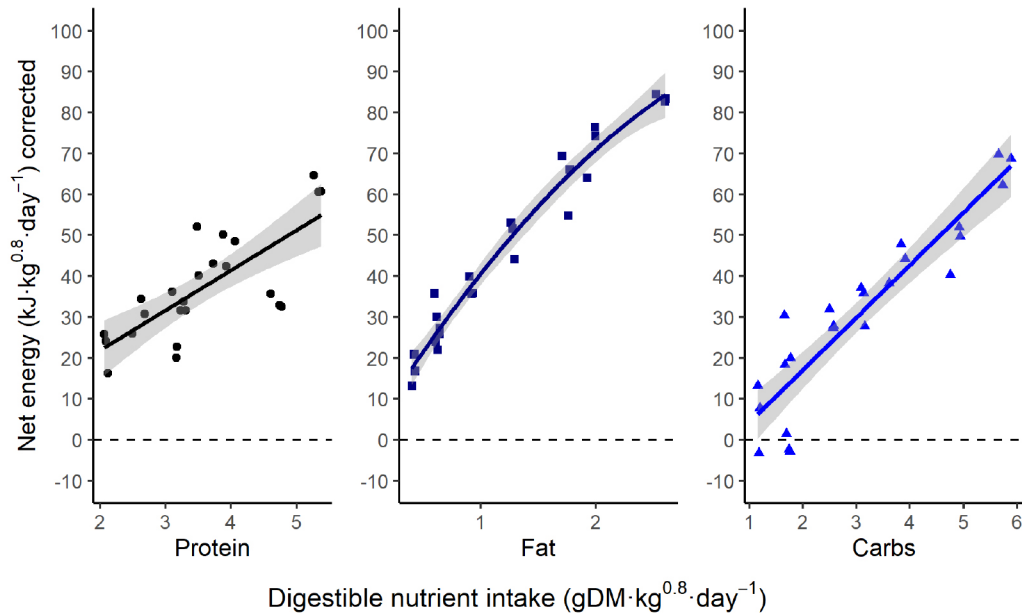


Figure 1. Isolated contribution of each macronutrient to the net energy of grass carp.

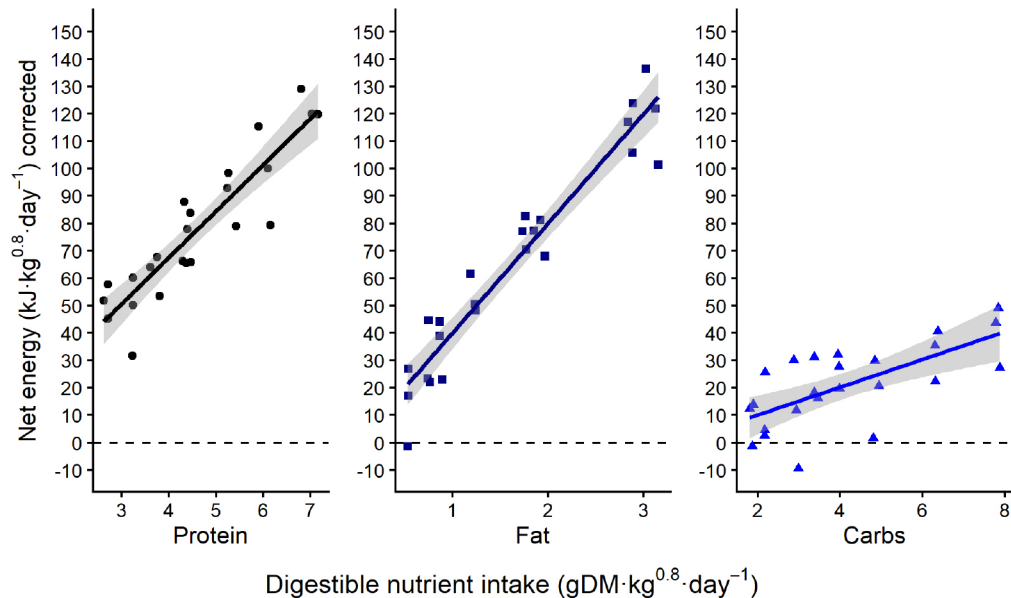


Figure 2. Isolated contribution of each macronutrient to the net energy of African catfish.

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EVALUATION OF DIFFERENT DIETARY DOSES OF AN ANTI-MYCOTOXIN ADDITIVE ON THE PERFORMANCE OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

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Introduction

Shrimp farming often suffers from high mortalities and poor growth. Mycotoxins are one of the contributing factors for these losses, however, they are often underestimated and rarely monitored in the aquaculture industry.

Driven by the need to reduce feeding costs associated with the increasingly high price of fishmeal, the industry has transitioned toward more cost effective and sustainable, plant-based protein sources. However, as these plant-derived ingredients progressively replace marine proteins in aquafeeds, the risk of shrimp exposure to fungal contaminants has intensified, making mycotoxin prevalence a critical challenge in modern aquaculture management. In this context, fumonisins are mycotoxins produced by fungi of the genus *Fusarium*, which are highly prevalent in corn and its by-products. In shrimp, documented effects include immunosuppression, multi-organ damage (particularly to the hepatopancreas) and degradation of muscle tissue (Oliveira et al., 2020). Collectively, these factors lead to significant reductions in survival rates, as well as in growth and overall production yield.

Consequently, implementing nutritional strategies such as the use of dietary anti-mycotoxin additives is an efficient approach to mitigate their toxic effects and enhance the overall survival and growth performance of shrimp.

This study evaluated the efficacy of a commercial anti-mycotoxin additive (AMA) specific for aquatic species against the adverse effects of fumonisins and its impact on the performance of Pacific white shrimp (*Litopenaeus vannamei*).

Materials and Methods

An anti-mycotoxin additive (AMA) was tested to assess its efficacy and determine the best dose for Pacific white shrimp. Four treatments (0, 1, 2, and 3 g AMA kg⁻¹ diet) were randomly allocated in 12 aquaria during larval rearing (20 days), followed by 12 fibreglass tanks during the subsequent grow-out phase (90 days). Both stages were conducted within controlled Recirculating Aquaculture System (RAS) conditions. To simulate natural contamination, standard shrimp feed was mixed with 10 % moldy corn meal, resulting in chronic low-level exposure to fumonisins, as confirmed by high performance liquid chromatography analysis.

The following performance parameters were evaluated: feed conversion, protein efficiency, survival, and growth. Differences between treatment groups were analyzed using a one-way analysis of variance (ANOVA) to determine the effects of the anti-mycotoxin additive, followed by Tukey's HSD test for post-hoc mean comparisons.

Results

During the larval rearing phase (Day 1–20), dietary supplementation with AMA significantly enhanced structural development. The addition of the additive resulted in increased final length and length gain ($p < 0.05$) across all treated groups compared to the control. While survival rates and weight gain remained statistically similar to the control during this initial short-term stage, the positive effects of the additive became more pronounced during the subsequent grow-out phase (Day 21–111). Performance indicators showed significant improvements in survival rate and feed efficiency ($p < 0.05$), while growth rates exhibited a positive numerical trend during the grow-out period. The inclusion of AMA resulted in increased survival from 27.3% in the control group to 60.3% in supplemented groups. Feed utilization was also optimized ($p < 0.05$), with the Feed Conversion Ratio (FCR) improving from 2.3 in the control to below 1.5 in all supplemented groups. Simultaneously, the Protein Efficiency Ratio (PER) showed an increasing trend with higher dosages reaching levels at least 1.5 times higher than the control ($p < 0.05$).

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Discussion

The administration of the AMA significantly improved growth and overall health by adsorbing directly feed contaminants, and reducing their toxicity through bio-protective and postbiotic mechanisms. During larval rearing, AMA increased length gain, while in the 90-day grow-out phase it doubled survival rates and enhanced feed efficiency. These benefits are attributed to the synergistic action of the AMA ingredients (selected clays, phytogenics like curcumin, and post-biotic components) that mitigated oxidative stress and bolstered the shrimp's immune response.

Conclusion

In conclusion, the tested AMA improved both larval rearing and grow-out performance between 1 and 3 g AMA kg⁻¹ diet, with optimal responses around 2 g AMA kg⁻¹ diet for the grow-out stage. These findings provide valuable insights by expanding our understanding of how mycotoxins impact farmed crustacean species. Furthermore, this research highlights the potential of novel additives as vital tools for modern aquaculture, serving as a robust foundation for future trials evaluating varying feeding regimes and stocking densities in commercial outdoor pond environments.

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EVALUATION OF DIFFERENT DIETARY DOSES OF AN ANTI-MYCOTOXIN ADDITIVE ON HEALTH STATUS AND IMMUNE RESPONSE OF PACIFIC WHITE SHRIMP (*Litopenaeus vannamei*)

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Introduction

High mortality and poor growth in shrimp farming are often linked to mycotoxin exposure, particularly due to the increasing use of plant-based proteins. Fumonisin, commonly found in corn-based feed, can cause immunosuppression, hepatopancreatic damage, and muscle necrosis in Pacific white shrimp (*Litopenaeus vannamei*). Previous studies suggest that anti-mycotoxin additives (AMA) can mitigate these effects and improve health and performance. This study aimed to evaluate the efficacy of a commercial AMA in counteracting chronic low-level fumonisin toxicity.

Materials and Methods

The AMA was tested at four inclusion levels (0, 1, 2, and 3 g kg⁻¹ feed) in a recirculating aquaculture system (RAS) during larval (20 days) and grow-out (90 days) stages. To simulate field conditions, diets were supplemented with 10% moldy corn meal, resulting in chronic low-level fumonisin contamination (46.6 µg kg⁻¹), confirmed by high-performance liquid chromatography (HPLC).

Health status was assessed through post-trial morphological examinations of the body and digestive organs, alongside the Hepatosomatic Index (HSI). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test.

A 14-day bacterial challenge was conducted to evaluate the AMA's effect on immune response. Shrimp were exposed to the median lethal dose (LD₅₀) of *Aeromonas hydrophila*, and survival and cumulative mortality were recorded.

Results

Morphological assessment showed that AMA-treated shrimp maintained normal coloration and gut integrity, whereas control shrimp exhibited pallor and intestinal inflammation. The hepatosomatic index (HSI) decreased with increasing AMA inclusion, reaching optimal values at approximately 2–3 g kg⁻¹. Histological analysis revealed normal hepatopancreatic structure in treated groups, in contrast to tissue damage and lipid accumulation in controls.

During the *Aeromonas hydrophila* bacterial challenge, AMA treatments delayed mortality onset and extended survival to 12 days, compared to 8.67 days in control groups ($p < 0.05$).

Discussion

Chronic low-level fumonisin exposure negatively affected shrimp health, as reflected by hepatopancreatic alterations, increased HSI, and reduced resistance to bacterial challenge. Dietary inclusion of the AMA mitigated these effects, improving tissue integrity, physiological condition, and survival under stress.

These benefits are likely explained by a multi-modal mechanism of action. Adsorbent materials such as bentonite can reduce mycotoxin bioavailability and have been associated with enhanced antioxidant activity and immune status (Kim et al., 2025). In parallel, phytochemical compounds derived from *Curcuma longa* and *Silybum marianum* have been shown to stimulate innate immune responses and reduce oxidative damage (Hasanthi et al., 2024). Additionally, postbiotic yeast components may contribute to improved redox balance and humoral immunity (Chotikachinda et al., 2008).

Conclusion

Dietary inclusion of the AMA effectively mitigated the negative effects of chronic low-level fumonisin exposure, improving hepatopancreatic health and resistance to bacterial challenge in *Litopenaeus vannamei*. The most consistent benefits were observed at 2 g kg⁻¹, particularly during the grow-out phase. These results support the use of AMA as a practical nutritional strategy to enhance shrimp robustness under commercial farming conditions.

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Acknowledgments

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COMPARATIVE STUDY OF *Salicornia ramosissima* DIETS FOR IMMUNE SUPPORT IN THERMALLY STRESSED RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Climate change-induced stressors can lead to chronic stress responses in fish, compromising their growth, immunity, and survival. To mitigate these vulnerabilities, the development of functional aquafeeds has emerged as a critical sustainable and prophylactic strategy. Halophytes like *Salicornia ramosissima* are highly promising candidates for these diets due to their immunomodulatory, anti-inflammatory, and genoprotective properties. In this study, two different extracts of *S. ramosissima* were added to experimental diets in order to produce fortified diets that bolster resilience against high-temperature stress—mimicking an environmental heatwave—in juvenile rainbow trout (*Oncorhynchus mykiss*).

Material and methods

Rainbow trout juveniles were distributed across 15 tanks in two recirculated aquaculture systems under either control (16 °C) or elevated (22 °C) temperatures. In both systems, triplicate groups of 20 fish were fed for four weeks either a control commercial-like diet or experimental diets featuring varying extracts and doses of *S. ramosissima*: Ext3_01, Ext3_05, XAD_01 and XAD_05. Post-trial sampling of three fish per replicate targeted blood and plasma for humoral analysis of immune parameters, liver for metabolic and oxidative stress evaluation, and head-kidney and brain for molecular profiling.

Results

Initial data revealed distinct humoral and oxidative stress profiles between the temperature groups, such as decreased plasma peroxidase levels, as well as decreased catalase activity at the elevated temperature (22 °C). Oxidative stress analysis also showed significant differences between two experimental diets, namely Ext3_01 and XAD_01 (Fig.1), which seem to suggest a mild dietary effect amongst the previously mentioned diets, however no significant statistical differences were observed between said diets and the control diet.

Also, preliminary analysis of the head-kidney may also suggest an apparent dietary effect at the HPI axis level between fish fed the control and their counterparts fed XAD_05 in relation to the *gr1* gene expression levels (Fig.2).

Conclusion

Ongoing analysis of remaining parameters, as well as pending final statistical analysis will hopefully elucidate the capacity of *Salicornia*-enriched diets to mitigate thermal stress in juvenile rainbow trout.

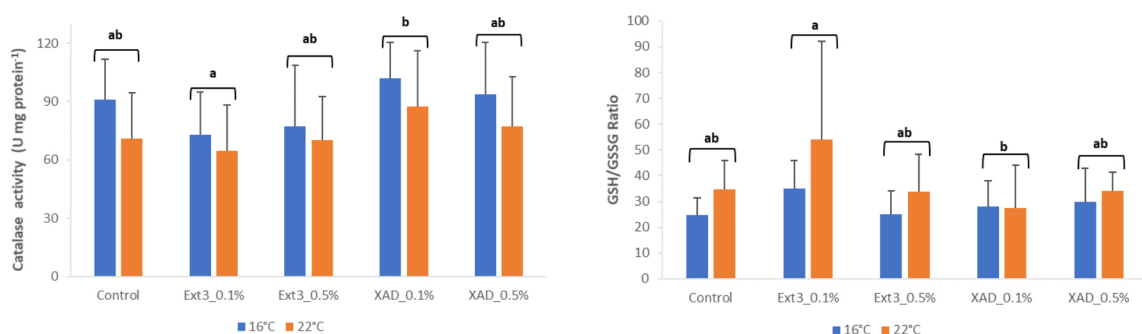


Fig.1 – Catalase activity and GSH/GSSG ratio from the liver of rainbow trout kept at 16 °C and 22°C and fed the experimental diets for four weeks (means $\pm$ SD). Different low-case letters stand for significant differences between dietary treatments (Two-wayANOVA followed by a Tukey post-hoc test, $p \leq 0.05$).

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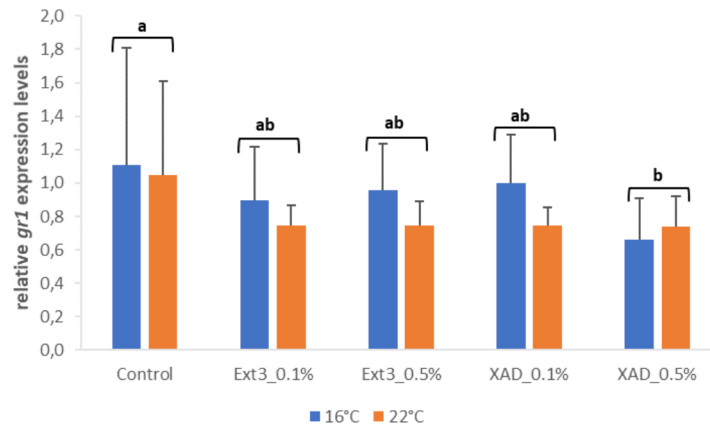


Fig.2 – Gene expression of glucocorticoid receptor 1 from head-kidney of rainbow trout kept at 16 °C and 22°C and fed the experimental diets for four weeks (means $\pm$ SD). Different low-case letters stand for significant differences between dietary treatments (Two-wayANOVA followed by a Tukey post-hoc test, $p \leq 0.05$).

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CIRCULAR PRODUCTION OF THE POLYCHAETE *Alitta virens* USING AQUACULTURE WASTE

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Introduction

The aquaculture intensification has led to an increase in nutrient-rich waste streams. Recent studies point to the potential for recycling the sludge from shrimp production as a feed source for low trophic organisms such as polychaetes. In this context, polychaetes can mineralize organic matter into high-value biomass, contributing to bioremediation and resource recovery. Polychaete biomass is rich in protein and essential fatty acids, being a valuable ingredient for aquafeeds. Therefore, the combination of waste management and the production of the nereid species *Alitta virens* is a potential method to enhance the circularity and sustainability of aquaculture systems. Thus, the aim of this work was to evaluate the use of aquaculture sludge as a potential feed source for the polychaete *A. virens* production.

Material and methods

To evaluate the growth performance of the polychaete *Alitta virens* when fed aquaculture waste, two trials were conducted using a recirculating aquaculture system (RAS). The system comprised 12 tanks (50 L), sump, biofilter, UV lamp, and chiller. Tanks were filled with aquarium sand to a substrate depth of 7 cm. Water quality parameters were maintained within suitable ranges for *A. virens* cultivation.

In both trials, sludge used as feed was collected from a shrimp farm in Germany, divided into Falcon tubes, and stored frozen. Prior to feeding, sludge was thawed under refrigeration, and the required quantities for each treatment were weighed 24 h in advance. Worms were fed once daily. The first trial lasted 96 days (initial weight: 2.83 ± 1.08 g, 80 animals/tank) and three treatments were evaluated: commercial shrimp feed (Feed), sludge (Sludge), and a combination of both (Sludge + Feed). The feeding rate was set at 0.5% of total biomass. The second trial lasted 67 days (initial weight: 1.23 ± 0.11 g, 110 animals/tank). Three treatments were tested based on increasing sludge feeding rates: 2% (Low), 4% (Medium), and 6% (High). At the end of each trial, all polychaetes were harvested, counted, and weighed, and growth indices were subsequently calculated.

Results and discussion

After 96 days of culture, *Alitta virens* fed exclusively with shrimp feed showed significantly higher growth performance than worms fed shrimp sludge or the mixed diet (Table 1). A similar trend was observed for the mean final weight. Survival remained high and did not differ significantly among treatments, indicating that shrimp sludge was suitable for maintaining worm viability even when used as the sole nutrient source.

In the second trial, where only sludge was supplied at different feeding rates, increasing sludge availability significantly improved growth performance (Table 2). Worms receiving the highest sludge ration achieved the greatest final biomass and mean final weight, while survival remained consistently high across all treatments. These results suggest that growth limitation under sludge-based diets was at least partially related to nutrient availability or feeding level rather than sludge acceptance itself.

The findings are consistent with previous studies demonstrating that nereid polychaetes can utilize aquaculture waste streams as a nutrient source in integrated multitrophic or circular aquaculture systems. Studies with *A. virens* and *Hediste diversicolor* similarly reported superior growth with formulated feeds but successful biomass production and high survival when aquaculture sludge was used as the sole feed source (Brown et al., 2011). The lower growth observed in sludge-fed worms is likely associated with reduced digestible energy and lower nutritional quality compared to commercial shrimp feed, particularly regarding protein and lipid availability. However, the ability of *A. virens* to survive and grow under sludge-based feeding demonstrates its potential for nutrient recovery and waste valorization in recirculating aquaculture systems (RAS) (Pettersen et al., 2025). These results show that polychaetes can contribute to circular aquaculture by converting nutrient-rich sludge into valuable biomass suitable for aquafeed ingredients.

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Table 1. Growth parameters of the polychaete *Alitta virens* after 96 days of culture being fed with feed, sludge or a mix of both (trial 1). Data are a mean $\pm$ SD. One-way ANOVA, $p < 0.05$.

	Feed	Sludge	Sludge + Feed	<i>p</i> -value
Final biomass (g)	298.3 $\pm$ 14.7 ^c	140.2 $\pm$ 20.8 ^a	235.0 $\pm$ 22.8 ^b	<0.0001
Mean final weight (g)	4.6 $\pm$ 0.4 ^c	2.1 $\pm$ 0.1 ^a	3.5 $\pm$ 0.1 ^b	<0.0001
Survival (%)	80.9 $\pm$ 9.1	81.6 $\pm$ 9.9	83.4 $\pm$ 5.6	0.9298

Table 2. Growth parameters of the polychaete *Alitta virens* after 67 days of culture being fed with sludge at different feeding rates (trial 2). Data are a mean $\pm$ SD. One-way ANOVA, $p < 0.05$.

	Low	Medium	High	<i>p</i> -value
Final biomass (g)	96.5 $\pm$ 11.3 ^a	100.7 $\pm$ 7.1 ^{ab}	116.0 $\pm$ 6.0 ^b	0.0231
Mean final weight (g)	0.90 $\pm$ 0.14 ^a	0.98 $\pm$ 0.05 ^{ab}	1.09 $\pm$ 0.06 ^b	0.0440
Survival (%)	96.8 $\pm$ 3.7	93.0 $\pm$ 4.5	96.4 $\pm$ 1.8	0.2641

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FROM VULNERABLE POST-LARVAE TO RESILIENT JUVENILES: ALGAE-ENRICHED FUNCTIONAL DIETS IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

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Introduction

European seabass (*Dicentrarchus labrax*) is a cornerstone species of Mediterranean aquaculture, with growing production driven by strong market demand. However, its early-life stages remain a critical vulnerability for the sector. Larvae display limited physiological capacity to cope with environmental fluctuations, handling procedures, and opportunistic pathogens, resulting in high sensitivity and variable survival. Ensuring that these fragile larvae develop into robust juveniles is therefore essential to improve production efficiency and sustainability. Functional diets have emerged as a promising strategy to enhance robustness during these early developmental stages. By incorporating bioactive compounds able to modulate immune, antioxidant, and stress-related pathways, functional diets can support the maturation of key physiological systems and improve resilience during common production stressors. Among functional ingredients, algae stand out due to their rich biochemical profile. Sulfated polysaccharides stimulate innate immunity, carotenoids and phenolic compounds enhance antioxidant defenses, and polyunsaturated fatty acids contribute to membrane stability and inflammatory regulation. These mechanisms can collectively reduce oxidative damage, modulate endocrine stress responses, and strengthen mucosal barriers. The objective of this work is to evaluate the potential of algae-based functional diets to enhance robustness European seabass during the early life-stages, particularly regarding stress modulation and resistance to a pathogen challenge.

Materials and Methods

Two sequential experimental trials were conducted at IPMA facilities to assess the effects of algae-based functional diets on the robustness of European seabass during early development. In Trial 1, dietary treatments consisted of a commercial-like control diet and three functional diets containing different combinations of *Euglena* sp., *Ulva* sp. and *Tetraselmis* sp. biomasses. In Trial 2, dietary treatments included a commercial diet, a commercial-like control diet and three functional diets containing combinations of *Ulva* sp., *Tetraselmis* sp. and *Chlorella* sp. biomasses. Fish from both trials (Trial 1: 83 days after hatching (DAH); Trial 2: 108 DAH) were reared in triplicate 300-L cylindroconical tanks under controlled zootechnical conditions and fed ad libitum with experimental microdiets for one week. Water quality parameters were kept within optimal ranges for the species, and routine husbandry ensured stable rearing conditions throughout the trial. Fish were then subjected to an acute air-exposure challenge to evaluate physiological stress responsiveness prior to bacterial infection. Fish were subsequently infected with *Pasteurella damsela* subsp. *piscicida* by bath exposure to evaluate the effect of dietary treatments on seabass disease resistance over a 14-day period. Fish sampling occurred at the end of the feeding period (before stress), 3 hours after stress and 24 hours after pathogen infection to analyse stress, immune and antioxidant-related enzymes and gene expression. Survival during the bacterial challenge was analysed using Kaplan–Meier curves.

Results and Discussion

No significant differences were observed on fish growth performance or survival during the experimental period of both trials. However, algae-based functional diets demonstrated measurable effects on stress resilience and pathogen resistance, although the magnitude of responses varied among trials and dietary treatments. In trial 1, no significant differences were observed between dietary treatments for stress (e.g. post-stress cortisol) and antioxidant-related biomarkers (CAT, SOD and GPX), but a blend composed of *Euglena*, *Ulva* and *Tetraselmis* biomasses led to an increase in the expression of immune-related genes (e.g. CCR3, CXCR4 and IL1 β). Survival analysis following stress and pathogen infection revealed no significant differences among treatments. Results obtained in the second trial so far showed no significant differences for fish mortality following the air exposure stress test (overall survival of 89 %). However, results from the pathogen challenge

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revealed that a combination of *Chlorella*, *Ulva* and *Tetraselmis* significantly reduced mortality risk (final value:56 %) in comparison to the commercial (final value:76 %) and the commercial-like control diet ($p=0.022$; final value: 69%). In addition, a blend of *Chlorella* and *Ulva* showed a strong trend toward improved survival ($p=0.067$) in comparison to the commercial-like control. Overall, results from both trials demonstrate that algae blends can contribute to improved stress and pathogen resilience during the early life stages of European seabass. Determining species combinations and optimal inclusion levels is critical for modulating physiological responses, as each algal biomass provides a distinct profile of nutrients and bioactive compounds. Therefore, this work supports the integration of algae biomasses into commercial microdiets as a sustainable strategy to reduce mortality during critical production phases in aquaculture.

Acknowledgments

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PEPTIDE-FORTIFIED FUNCTIONAL FEEDS FOR MARINE FISH: FROM *IN SILICO* SCREENING TO MULTI-SPECIES FORMULATION

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Introduction

Bioactive peptides have emerged as promising functional ingredients to mitigate stress responses in fish. These molecules, derived from controlled protein hydrolysis or naturally present as defined neuropeptides, can modulate key physiological pathways involved in stress resilience, including immune activation, antioxidant defense, neuroprotection and nociception. Their biochemical nature makes them highly suitable for aquaculture: peptides are generally regarded as safe, biodegradable, orally active and rapidly absorbed in the digestive tract lumen. Within this context, the FORTIFISH project was established to advance the development of peptide-fortified functional feeds for early-life stages of marine fish. Since it is not feasible to experimentally test *in vivo* the vast number of peptide sources available on the market, an *in silico* screening approach emerged as a high-throughput screening tool to prioritize raw materials with predicted bioactivity and relevance for stress modulation. This screening framework provides a rational foundation to select peptide-rich ingredients to be incorporated into functional diets targeting stress resilience in marine fish larvae, where nutritional knowledge is still limited and physiological sensitivity is high. Moreover, larvae start exogenous feeding without a differentiated stomach, influencing how dietary protein is digested and absorbed, a trait that needs to be taken into account when assessing the bioactivity potential of peptides during the early life stages of fish. This work aims to describe the workflow developed within the FORTIFISH project to design peptide-fortified functional feeds for marine fish, detailing how *in silico* triage was used to select peptide-rich raw materials and how these were incorporated into species-specific microdiets for seabass, seabream and zebrafish, establishing a multi-species formulation framework for functional feed development.

Materials and Methods

The methodological workflow developed within the FORTIFISH project combined *in silico* peptide-release prediction with ingredient-level scoring to support the design of peptide-fortified functional feeds for marine fish. Protein profiles of common aquafeed ingredients were compiled, including protein content, taxonomic origin and relative abundance of major proteins. Protein sequences were retrieved from UniProtKB using a standardized query pipeline that prioritized reviewed entries and included unreviewed sequences when necessary, followed by manual curation to ensure correct taxonomic matching and removal of low-quality entries. Simulated digestion, including missed cleavages to simulate a non-perfect digestion, was performed using enzyme-specific cleavage rules to mimic industrial hydrolysis and fish gastrointestinal digestion: hydrolysates underwent a two-step simulation (alcalase followed by pepsin, trypsin and chymotrypsin), whereas intact proteins were subjected to a single digestion step reflecting fish digestive physiology. For each protein isoform, all peptides generated through *in silico* digestion were quantified and classified, and protein-level metrics were calculated, including total peptide yield, peptide length distribution and normalized scores adjusted for molecular weight and protein abundance. Ingredient-level metrics were then obtained by aggregating protein-level values using abundance-weighted means, providing a standardized comparison across ingredients with different protein compositions. Following this selection process, the prioritized peptide-rich ingredients were incorporated into larval microdiets for seabass, seabream and zebrafish, produced at SPAROS' Feed Technology Platform to ensure appropriate particle engineering and nutritional profiles for early-life feeding.

Results and Discussion

The *in silico* digestion generated extensive peptide profiles across all ingredients, revealing clear and consistent patterns. Hydrolysates systematically produced more peptides than intact proteins, confirming the impact of the additional enzymatic step. For example, hydrolysed salmon and anchovy proteins yielded broader peptide spectra than their non-hydrolysed counterparts, while insect meals such as black soldier fly and mealworm also showed high peptide release due to the abundance of actin and myosin. Structural proteins, particularly collagen and myosin, were among the strongest contributors to peptide generation across fish meals, gelatine sources and poultry hydrolysates. Dairy proteins also stood out as relevant contributors: casein produced abundant short peptides due to its cleavage-prone regions, and whey proteins (notably β -lactoglobulin and α -lactalbumin) generated diverse peptide profiles enriched in residues associated with predicted anti-stress activity. Hydrolysed variants of both casein and whey further increased the proportion of short

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bioactive fragments. Across ingredients, the screening consistently identified short peptides enriched in aromatic and basic residues as the peptide signatures with the highest predicted anti-stress potential. These peptides were particularly frequent in collagen-rich ingredients, insect meals, and dairy proteins, and were more abundant in hydrolysates due to the additional enzymatic step. Ingredients generating these favourable peptide profiles were therefore prioritized for larval formulation. In contrast, plant proteins (e.g., pea, soy, wheat) and microalgae (e.g., *Spirulina*, *Tetraselmis*) produced fewer peptides, reflecting shorter sequences and distinct amino acid compositions. From a formulation perspective, ingredients with high peptide-release potential and favourable peptide signatures are especially relevant for larval diets, where digestive capacity is limited and peptide absorption is rapid. Overall, the screening pipeline enabled the identification of peptide-rich ingredients suitable for incorporation into larval microdiets for seabass, seabream and zebrafish, produced at SPAROS' Feed Technology Platform.

Acknowledgments

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GENETIC VARIABILITY OF PERFORMANCE STABILITY WITHIN THREE POPULATIONS OF EUROPEAN SEA BASS (*Dicentrarchus labrax*) SUBJECTED TO DIFFERENT THERMAL REGIMES

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Introduction

In the context of climate change, sea surface temperature has already increased by 0.04°C per year between 1985 and 2006 in the Mediterranean Sea and, according to projections by the Intergovernmental Panel on Climate Change, it could rise by 1 to 2°C in the Atlantic Ocean and by 2.2 to 3.4°C in the Mediterranean Sea by 2080. Such thermal elevation is likely to strongly affect sea bass populations and, consequently, aquaculture production. However, these effects may vary depending on the genetic origin of the populations: Atlantic (AT), Western Mediterranean (WM), and Eastern Mediterranean (EM), which have evolved under different environmental conditions.

In response to changes in their thermal environment, fish must adapt in order to limit the negative effects of these variations. A key component of this adaptation is robustness, defined as the ability to maintain production potential across a wide range of environmental conditions without compromising health and welfare (Knap, 2005). In this context, the stability of growth performance within a population exposed to different temperatures is an important criterion of robustness, since a fish exhibiting stable growth across temperatures will maintain production potential over a broad range of environments. This stability can be studied using the “LnVar” approach, an indicator that describes the variability of individual performance over time to assess organism sensitivity to external fluctuations. This indicator has been shown to exhibit genetic variability in several terrestrial and aquatic species, opening perspectives for selective breeding of more robust animals.

Materials and Methods

European seabass from the AT, WM, and EM populations were reared in mixed groups under three distinct thermal regimes representative of the seasonal variations in their areas of origin: a relatively cold regime for the Atlantic (rAT), an intermediate regime for the Western Mediterranean (rWM), and a warmer regime for the Eastern Mediterranean (rEM) (Crestel et al., 2025).

At an average body weight of 10 g, 5,148 fish were individually tagged, allowing individual monitoring of performance traits (weight, length, growth, etc.) over three years through biometric measurements taken every six weeks. For each individual, the stability of the studied traits, LnVar, was calculated as follows :

$$\ln(\text{variance}_j) = \ln\left(\frac{\sum_{i=1}^{n_j} (x_{ij} - \bar{x}_j)^2}{n_j - 1}\right), \text{ where } x_{ij} \text{ is the deviation of measurement } i \text{ relative to the cohort of}$$

individual j for the studied trait, and $\bar{x}_j$ is the mean deviation of individual j for that trait. Two additional indicators, skewness and autocorrelation, were calculated to complement this study (Berghof et al., 2019). These indicators were calculated using body weight (BW) of Daily Growth Coefficient (DGC) as base traits.

Individuals were genotyped using the Axiom™ 57K SNP DlabChip array in order to estimate the genetic parameters of the measured traits and their stability.

Results

The LnVar classified fish as more or less stable in response to temperature variations, but it does not allow discrimination between high- and low-performing fish. Autocorrelation and skewness helped to characterize the temporal dynamics of fluctuations when individuals were not stable. Heritability was 0.14 ± 0.03 for both BW LnVar and BW skewness, while it was 0.00 ± 0.00 for BW autocorrelation. The genetic correlations between BW LnVar and BW at two years was 0.72 ± 0.15 and -0.29 ± 0.14 between BW LnVar and BW skewness. With DGC as base trait, h^2 was 0.34 ± 0.03 for LnVar, 0.15 ± 0.03 for skewness and 0.0 ± 0.0 for BW autocorrelation. Other analysis are currently running taking into accounts other effects.

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Discussion

These combined indicators help better understand fish growth profiles under thermal variation. InVar appears to be the most relevant indicator, as it both effectively discriminates fish according to their stability and can be used in selective breeding, given its moderate heritability, especially with DGC as the base trait.

This study, currently in progress, will also determine how many measurements are required to obtain a reliable estimate of individual InVar. Such information is important for breeding programs, to assess how InVar can be meaningfully estimated. At a later stage, we will seek to establish correlations of InVar with production traits and operational welfare indicators.

Acknowledgment

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LIVING ON THE EDGE: PHYSIOLOGICAL RESPONSES OF *Octopus maya* TO THE PEJUS RANGE FROM EMBRYO TO ADULTHOOD

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Introduction

Rising ocean temperatures affect marine ectotherms, including *Octopus maya*, an endemic species of the Campeche Bank with ecological and fisheries importance (Pörtner et al., 2017; Solís-Ramírez, 1997). Its thermal optimum is 24–26°C, while temperatures $\geq 28^\circ\text{C}$ induce physiological and metabolic stress, and $>30^\circ\text{C}$ negatively affect reproduction and offspring development (Ángeles-González et al., 2020; Caamal-Monsreal et al., 2016; Juárez et al., 2016). However, most studies have focused on isolated life cycle stages, with little multi-stage integration (Domínguez-Castanedo et al., 2023; Vargas-Abúndez et al., 2022). Therefore, the aim is to evaluate how maternal exposure to thermal stress influences the physiological response of embryos, juveniles, pre-adults and adults under 28°C , analyzing metabolism, oxidative stress, immunity and reproduction, to understand the thermal resilience of the species to ocean warming.

Materials and Methods

Sixty-seven adult *Octopus maya* captured in Sisal, Yucatán (July 2025) were used. Of these, 53 were exposed to thermal stress (28°C) for one month, and 14 males were maintained at an optimal temperature (24°C). Stressed females were mated with control males. Reproductive parameters (spermatophores, spermatozoa, gonadosomatic and hepatosomatic indices, number of eggs, clusters, and fertility), as well as metabolism (MO_2), antioxidant defense, oxidative damage, and energy reserves were evaluated in subgroups of both males and females. The eggs were incubated at 28°C . A total of 255 embryos were analyzed for development, survival, metabolism, antioxidant status, and yolk. Subsequently, 245 juveniles were reared for seven months to pre-adulthood under controlled conditions. In juveniles and pre-adults, aerobic metabolism, maximum capacity (TIMR), thermal aerobic potential, critical temperatures, energy status, immunity, and oxidative stress were measured, with random selection at each stage.

Preliminary Results

Preliminary results indicate that embryonic survival of *Octopus maya* at 28°C was close to 90%, with mortality associated with malformations and abnormal yolk. A heterogeneous response was observed in the antioxidant system, with increased protein carbonylation and low esterase activity, suggesting oxidative stress, although interpretation is still limited. In later stages, the survival of juveniles and pre-adults progressively decreased. Critical temperatures were similar between stages ($\text{TC}_{\text{max}} \sim 37^\circ\text{C}$; $\text{TC}_{\text{min}} 10\text{--}12^\circ\text{C}$). Differences were observed in energy indices and antioxidant response, with a greater contribution from lipid damage in juveniles and pre-adults, and significant differences in esterase activity between stages (PERMANOVA, $p = 0.001$). In reproduction, 44% of females produced fertile spawns, with high variability in egg production; males showed sperm viability of $\sim 93\%$. Taken together, the data suggest effects of heat stress at various physiological levels, but all results are preliminary and require further analysis before definitive conclusions can be drawn.

Discussion

Exposure of *Octopus maya* to 28°C elicited distinct physiological responses throughout its life cycle. In embryos, the high survival rate ($\sim 90\%$) suggests general tolerance to heat stress, at least at this stage, although the presence of malformations and yolk abnormalities indicates sublethal effects on development. Physiologically, signs of oxidative imbalance were observed, evidenced by variations in antioxidant enzymes and an increase in protein damage markers. In juvenile and pre-adult stages, survival decreased with exposure time, suggesting possible cumulative effects of heat stress. Furthermore, ontogenetic differences in the antioxidant response and lipid peroxidation suggest variable susceptibility depending on the developmental stage. Thermal tolerance values remained relatively similar across stages, while reproduction showed a reduction in females with fertile spawning, despite high sperm viability in males, suggesting a potentially greater impact on the female component. Taken together, the results indicate that 28°C induces sublethal effects and developmental-stage-dependent physiological responses in *Octopus maya*. Since some analyses are still in progress, these interpretations should be considered preliminary.

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STRUCTURAL AND METABOLIC CHANGES IN THE INTESTINE AND THE PYLORIC CAECA OF RAINBOW TROUT *Oncorhynchus mykiss* UNDER DIFFERENT GROWTH CONDITIONS

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Introduction

The gastrointestinal (GI) tract of fish plays a key role in nutrient absorption, energy metabolism, and growth, and exhibits significant plasticity in response to food availability. In salmonids such as rainbow trout (*Oncorhynchus mykiss*), the intestine and pyloric caeca are central to digestion and can rapidly adjust to feeding conditions. Starvation is typically associated with reductions in gut mass, mucosal thickness, and absorptive surface area, whereas continuous feeding promotes hypertrophy and increased digestive capacity. However, it remains unclear whether these structural changes are accompanied by corresponding shifts in metabolic capacity at the tissue level.

The present study aimed to investigate how contrasting feeding regimes influence both the morphology and metabolic function of digestive tissues in rainbow trout.

Material and Methods

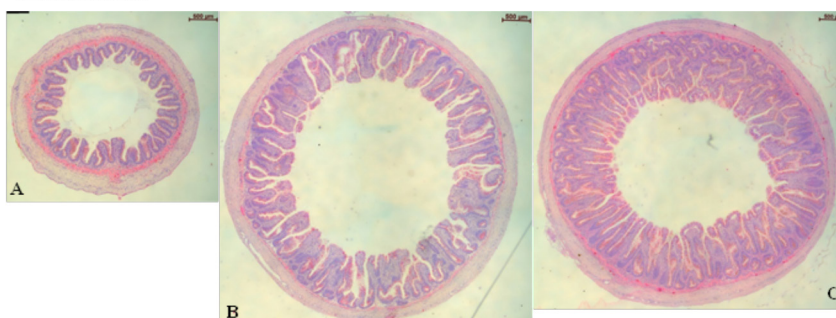
The experiment was conducted with rainbow trout reared in a recirculating aquaculture system. Fish were assigned to one of three feeding treatments: 28-day starvation, intermediate feeding at 1.4% body weight per day, and *ad libitum* feeding at 2.5% body weight per day. To minimise size-related variation, all fish were sampled at a target body mass of approximately 250 g.

The gastrointestinal tract was dissected, and the anterior intestine and pyloric caeca were collected for analysis. Structural changes were assessed through histology and somatic indices. Transmission electron microscopy (TEM) was used to visually examine ultrastructural features such as mitochondrial density. Metabolic capacity was evaluated through tissue respirometry to measure mass-specific oxygen consumption, and citrate synthase activity assays as an indicator of mitochondrial density and aerobic capacity. Statistical analyses were performed to determine differences among treatments.

Results

Distinct differences in gastrointestinal structure were observed among feeding treatments. Starved fish showed clear atrophy of digestive tissues, including reduced organ mass, thinner intestinal mucosa, and decreased mucosal complexity. In contrast, fed fish displayed hypertrophic responses, with increased mucosal thickness and more developed intestinal structures in both the intestine and pyloric caeca.

Figure 1. Stitched cross-section images of the anterior intestine from an individual of the starved (A), intermediate (B), and *ad libitum* (C) treatments.



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Despite these structural changes, metabolic measurements remained largely unchanged. Mass specific oxygen consumption (QO_2) in the anterior intestine did not differ significantly between treatments, although pyloric caeca showed reduced rates in starved fish. Citrate synthase activity also showed no significant variation, indicating maintained mitochondrial density and aerobic capacity. Ultrastructural observations supported these findings, with no major differences in mitochondrial abundance or cellular integrity between groups.

Discussion

The results demonstrate a clear decoupling between structural and metabolic responses in the gastrointestinal tract of rainbow trout. While starvation induced pronounced atrophy of digestive tissues, including reductions in mucosal thickness and organ mass, metabolic capacity at the cellular level remained largely stable. This suggests that short-term food deprivation primarily affects tissue structure rather than intrinsic mitochondrial function, supporting an energy-saving strategy where digestive tissues are reduced in size while maintaining readiness for rapid recovery upon refeeding.

The structural plasticity observed in both the intestine and pyloric caeca aligns with changes in growth rate and somatic indices, indicating that digestive investment is closely linked to nutrient intake. However, the absence of consistent changes in citrate synthase activity and oxygen consumption suggests that metabolic regulation occurs primarily through adjustments in tissue mass rather than mass-specific metabolic capacity.

The citrate synthase assay may have lacked specificity, potentially masking treatment-related differences, while limited replication in transmission electron microscopy and variability in tissue respirometry may have reduced the ability to detect subtle metabolic changes.

Overall, these findings highlight the resilience and efficiency of gastrointestinal adaptation in rainbow trout, where structural remodelling enables energy conservation without compromising cellular metabolic function, with important implications for aquaculture feeding strategies.

Acknowledgment

I would like to express my eternal gratitude to Denmark's Technical University (DTU) for providing me with the opportunity to do this master's thesis!

SUSTAINABLE SOLUTION FOR GEOSMIN AND 2-MIB REMOVAL IN STURGEON FARMING AND CAVIAR PRODUCTION USING ADVANCED OXIDATION

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Introduction

Fish reared in recirculating aquaculture systems often accumulate off-flavor compounds, such as geosmin (GSM) and 2-methylisoborneol (2-MIB), negatively affecting product quality. Traditional depuration with a constant supply of fresh water is effective but water-intensive. Moreover, this lengthy process is potentially suboptimal for fish welfare. The patented advanced oxidation process (eAOP®) technology (Exiton Clean, USA) can offer a sustainable alternative by rapidly degrading off-flavor molecules during depuration, reducing water use and shortening depuration time (Kropp et al., 2022). This study validates the safety and effectiveness of eAOP in sturgeon depuration for caviar production.

Materials and methods

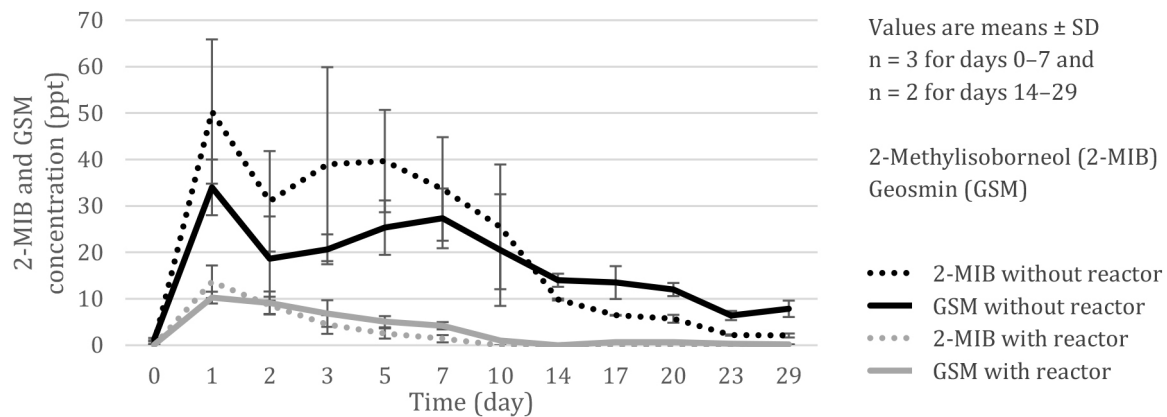
A depuration experiment was conducted in six 1.3 m³ tanks: triplicate control tanks equipped with a circulation pump and oxygenation, and triplicate tanks equipped with a circulation pump coupled to an eAOP reactor and oxygenation. Each tank hosted 32 fish (50 kg m⁻³) of male Danube sturgeons (*Acipenser gueldenstaedtii*) of ~1.9 kg individual body weight. Fish were not fed during the trial. Water temperature was maintained at 12°C. Water quality parameters were monitored daily and unionized NH₃ concentration was kept under 0.05 mg l⁻¹ by daily water changes. A steady H₂O₂ concentration of 10 mg l⁻¹ was maintained in the tanks with eAOP reactor to sustain the advanced oxidation reaction.

Water samples were collected daily and GSM and 2-MIB concentrations were determined by online extraction on a specific adsorbent, followed by gas chromatography with flame ionization detector (GC-FID). At day 0 and after 0.5, 1, 2, 3 and 4 weeks of depuration, fillet tissue and gill arches were sampled from three fish per tank to determine the GSM and 2-MIB concentrations in fillet tissue, and to detect unlikely gill damage due to chronic exposure to H₂O₂ respectively. GSM and 2-MIB concentrations in fillet tissue were determined by pressurized liquid extraction (PLE), clean-up using aluminum oxide and gel permeation chromatography, followed by analysis by gas chromatography–tandem mass spectrometry (GC–MS²). Macroscopic and histopathological analysis of eight most pronounced alterations (epithelial hypertrophy, epithelial hyperplasia, mucous cell alterations, chloride cell alterations, epithelial lifting, necrosis, hyperemia, angiectasia, inflammation) of the gill filaments were performed by two experts according to Marinovic et al. (2021).

GSM and 2-MIB concentrations in water samples decreased below 10 ppt after day 23 and day 14 respectively in tanks without eAOP reactor, due to the daily water changes. In tanks with eAOP reactor, this concentration was already obtained after two days (Figure 1). Preliminary results of fillet samples show that GSM and 2-MIB concentrations decrease faster in fillets from fish kept in tanks with eAOP reactor, demonstrating the effectiveness of the eAOP reactor.

Macroscopic examination demonstrates that gill lesions were largely absent. Almost all sampled gill arches exhibited no lesions and only five of 90 sampled fish, all from tanks without eAOP reactor, exhibited very light lesions. No moderate or severe macroscopic abnormalities were observed. Also, histopathological analysis did not show a significantly increased histopathological index of the gill arches of fish kept in tanks with eAOP reactor, demonstrating the safety of the eAOP reactor.

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Figure 1: GSM and 2-MIB concentrations in water samples

Acknowledgment

This research was funded by the European Union & the Flemish government through the ‘European Maritime, Fisheries and Aquaculture Fund (EMFAF)’ and ‘het Financieringsinstrument voor de Vlaamse Visserij en Aquacultuursector (FIVA)’ grant for the Kavixton project.

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EFFICIENCY OF A MIXED BIOREACTOR SYSTEM FOR NUTRIENT AND WATER QUALITY CONTROL IN WHITE SHRIMP (*Penaeus vannamei*) FARMING IN BFT SYSTEMS

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The objective of this work was to evaluate the efficiency of a mixed bioreactor of piston flow and aerobic and anaerobic phases to solve the main operational limitations of pure Biofloc technology (BFT) systems in the long term: the accelerated consumption of alkalinity and the uninterrupted accumulation of nitrates and phosphates. A Control (C) group was evaluated against a Treatment (T).

The results showed reductions of 38.1% in ammonium, 36.8% in nitrate and 24.9% in phosphate. Nitrate levels showed the highest average concentrations in the Control system with 63.25 mg/L, compared to the Treatment system where an average concentration of 39.96 mg/L was observed (Figure 1). Likewise, the average ammonium concentration decreased from an initial value of 0.21 mg/L in the Control to a value of 0.13 mg/L in the treated system. On the other hand, alkalinity had a stabilizing effect with an increase of 4.8%, going from an average of 150.08 mg/L in the Control to 157.33 mg/L in the Treatment (Figure 3).

The incorporation of the mixed bioreactor proved to be an efficient technical solution to overcome the chronic limitations of BFT systems. By stabilizing alkalinity and significantly reducing the buildup of critical nutrients such as nitrate and phosphate. This technology is confirmed as a viable, scalable pilot experiment to optimize water quality control in the intensive culture of *Penaeus vannamei*.

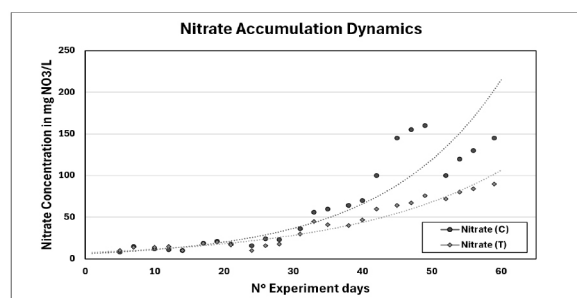


Figure 1: Dynamics of nitrate accumulation (mg NO₃/L) as a function of time for the control group (C) and treatment (T), throughout the 60 days of the experiment.

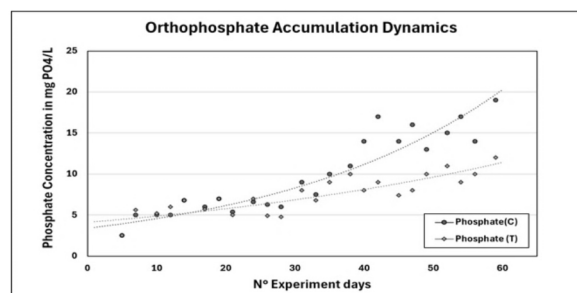
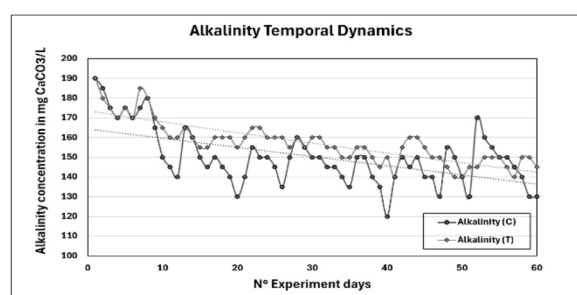


Figure 2: Dynamics of Phosphate accumulation (mg PO₄/L) as a function of time for the control group (C) and treatment (T), throughout the 60 days of the experiment.



experiment.

Figure 3: Temporal dynamics of total alkalinity (mg CaCO₃/L) as a function of time for the control group (C) and treatment (T) throughout the 60 days of experiment.

A COLLECTIVE INITIATIVE FOR DOMESTICATION AND GENETIC IMPROVEMENT OF AQUACULTURE INVERTEBRATES IN FRANCE

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Introduction

In France, the production of a few farmed bivalve species such as *Crassostrea [Magallana] gigas* and *Ruditapes philippinarum*, benefit from breeding programs operated by commercial hatcheries with the support of SYSAAF. Some programs have advanced to the implementation of genomic selection (Boudry et al., 2021). In 2024, this well-established hatchery sector produced 3.5 billion Pacific oysters spat and 1 billion of other molluscan species (Agreste, 2024). Hatcheries production has also been developed for the population enhancement for other species, such as *Pecten maximus* (Morgezen et al., 2016) consisting of mass release of hatchery-produced juveniles in the local naturally occurring population of great scallops (*Pecten maximus* L. and *Ostrea edulis* (Pouvreau et al., 2023). However, most initiatives lack of a proper management of broodstock's genetic diversity and interaction with wild populations.

The French aquaculture production of non-native shrimps and pawns, including *Macrobrachium rosenbergii*, *Marsupenaeus japonicus*, *Litopenaeus vannamei* and *L. stylirostris*, has been also established for several decades. Nevertheless, because no pedigree records have been maintained since the introduction of native broodstock, their current genetic status remains poorly characterised, highlighting the need to secure existing genetic resources currently maintained in captivity (Goyard et al., 2003).

Echinoderms, such as *Holothuria tubulosa*, *H. forskali* and *Paracentrotus lividus*, remain at an early stage of domestication. Because production relies on wild broodstock, genetic characterisation and monitoring are essential to support the sustainable development of these emerging, high-value species. Similarly, *Mytilus edulis*, *M. galloprovincialis* and *Cerastoderma edule* are not currently hatchery-produced in France, although international studies indicate potential for domestication and genetic improvement (Ajithkumar et al., 2025; Pampín et al., 2023) this species faces a significant threat from infectious disease outbreaks in both mussel farms and the natural environment over the past decade. Diseases caused by various pathogens, particularly *Vibrio* spp., have posed a significant challenge to the mussel industry. Genetic improvement of disease resistance can be an effective approach to overcoming this issue. Methods In this work, we tested genomic selection in the blue mussel (*Mytilus edulis*).

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Overall, invertebrate aquaculture sectors in France display variable levels of domestication and genetic management (Purcell et al., 2025) genetic and genomic approaches can be leveraged to enhance understanding of the diversity and complexity of these organisms, including the linkage between phenotype and genotype, and their adaptive and breeding potential. These approaches can inform processes ranging from the initial collection of wild broodstock to the ongoing use of genomic selection on domesticated lines. Due to the diversity in cultured fish species, small and medium enterprises (SMEs). This situation highlights the need to improve the characterisation, conservation and management of genetic resources in order to ensure the sustainability, resilience and continued development of these aquaculture sectors. To address these challenges, the collective public initiative TEHASIS was launched in 2026. It is based on a collaborative approach bringing together farmers, academic and technical stakeholders, covering mainland France, Ultra-Peripheral Regions (UPR) and Overseas Countries and Territories (OCT).

Objectives

TEHASIS aims to develop a strategic framework for the domestication and the genetic improvement of French invertebrates' aquacultures through an integrated approach combining stakeholder mapping, assessment of genetic management practices and knowledge transfer. The main objective is to co-design, for each species, a genetic roadmap for their domestication and genetic improvement to strengthen hatchery resilience, improve production quality and enhance adaptation to future challenges. In parallel, technical workshops, training sessions targeting professionals and public awareness are planned to improve social perception of domestication and genetic improvement of genetic resources in invertebrate's aquaculture.

Action plan

The first phase consists of an exhaustive inventory of commercial hatcheries of aquatic invertebrates operating in France. This inventory, now nearly complete, has identified over twenty species companies.

Three species-based working groups, "major selected shellfish" (Pacific oyster and Manila clam), "shrimps" and "others invertebrates" are created gathering farmers, academic and technical stakeholders. These working groups will provide shared and comprehensive synthesis on knowledge on genetic improvements for the most important species, and for special issues related to them. In parallel, a mapping of the stakeholders, and associated value chains will be conducted, to identify opportunities and weaknesses of these emerging new sectors, to design scenarios, and to qualify potential environmental, societal, socio-economic risks for the future roadmap of these values chain.

In parallel, a review of species- and topic-specific synthetic review will provide shared and comprehensive synthesis on knowledge on genetic improvements. Based on those findings, each working group will produce an operational roadmap identifying technological bottlenecks, research and innovation priorities, and the most appropriate genetic improvement strategies according to the biological, economic, organizational and regulatory characteristics of each sector.

Expected outcomes

By the end of the project, TEHASIS is expected to provide a road map to initiate domestication and genetic improvement within French invertebrate aquaculture hatcheries while increasing awareness among professionals of the benefits of genetic management and selective breeding. By promoting the adoption of genetic tools and concepts, the project aims to make these approaches more accessible, thereby enhancing the competitiveness, innovation capacity and resilience of the sectors.

TEHASIS also seeks to strengthen collaboration between farmers, scientific and technical stakeholders by establishing a national network dedicated to invertebrate aquaculture genetics and promoting knowledge exchange and innovation.

In the longer term, the project is expected to support the establishment of a sustainable public technical and scientific support framework for genetic management and breeding in these emerging productions. The knowledge generated, operational roadmaps and collaborative network developed through TEHASIS should also help identify sector-specific research priorities and stimulate future applied research and innovation projects.

Acknowledgment

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A HAPLOID RESOLVED, CHROMOSOME-SCALE GENOME ASSEMBLY OF EUROPEAN WHITEFISH (*Coregonus lavaretus* L.) REVEALS HIGH RETENTION OF DUPLICATED GENOME CONTENT

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Introduction

European whitefish (*Coregonus lavaretus*) is a member of Salmonids used for aquaculture, commercial fisheries, and conservation across northern and central Europe. It is valued for its fast growth, product quality, and adaptability to different production systems (Kankainen et al. 2016). However, the lack of a high-quality reference genome has limited the application of modern genomic tools in selective breeding and management. A major challenge however, is that like other salmonids (Macqueen and Johnston 2014) “plainCitation”: (Macqueen and Johnston 2014, it has experienced an ancient whole-genome duplication, and parts of the genome still retain duplicated regions (Pokharel et al. 2025). This makes it difficult to distinguish true biological duplication from technical redundancy during genome assembly. Resolving this issue is important, as incorrect handling of duplicated regions can reduce the usefulness of the genome for downstream applications such as marker discovery and genomic selection. In this study, we aimed to generate a high-quality, chromosome-scale, haplotype-resolved genome assembly for European whitefish and to clarify the extent of duplicated genome content.

Materials and Methods

A mature female European whitefish from the Finnish national breeding programme maintained by Luke (Finland, 2019 year class) was sequenced using PacBio HiFi technology (~152.8 Gb). To improve long-range genome assembly, Omni-C proximity ligation libraries were prepared from fin, liver, and muscle tissues and sequenced on an Illumina NovaSeq (~357 Gb). Genome characteristics, including size, heterozygosity, and ploidy structures, were estimated using k-mer-based approaches including GenomeScope2 (Ranallo-Benavidez et al. 2020), Jellyfish (Marçais and Kingsford 2011), and Smudgeplot (Ranallo-Benavidez et al. 2020). Multiple assembly strategies were tested using Hifiasm (Cheng et al. 2021) with different levels of duplicate purging to balance removal of redundant sequences while retaining biologically meaningful duplicated regions. Selected assemblies were scaffolded using Yaho (Zhou et al. 2023) with Omni-C data, and quality was assessed based on contiguity metrics and BUSCO (Manni et al. 2021) gene completeness.

Results

Genome analyses supported a diploid genome structure with substantial retained duplicated content consistent with salmonid whole-genome duplication. Approximately 45–60% of the genome showed signals of incomplete diploid resolution, reflecting retained duplicated regions rather than higher ploidy. Across different assembly strategies, consistent results were obtained for genome size and structure. The final selected assembly achieved chromosome-scale contiguity, with scaffold N50 values of ~68 Mb. The primary assembly size was ~3.09 Gb, with two alternative haplotypes of ~2.89 Gb and ~2.49 Gb. BUSCO completeness exceeded 99%, indicating a highly complete assembly.

Discussion

This study provides the first haplotype-resolved, chromosome-scale genome assembly for European whitefish focusing on separating the pure diploid and the duplicated regions. The results support a diploid genome structure with substantial retention of duplicated regions, reflecting the species’ evolutionary history rather than technical artifacts. So far, the single-nucleotide polymorphism (SNP) arrays used in modern aquaculture breeding programs are typically limited to the diploid part of the genome. This is a significant issue in a breeding context, as traits encoded in the non-diploid parts of the genome are currently not captured, and entire gene families potentially generated by the duplication event may be unreachable for breeders. The reference genome generated here provides a robust genomic resource for genomic selection, marker development, and population genetic analyses, supporting both fundamental research and development of genomic tools for European whitefish aquaculture and management.

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Acknowledgment

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GEOSMIN AND 2-METHYLISOBORNEOL EXPOSURE AFFECTS IMMUNE-RELATED GENE EXPRESSION IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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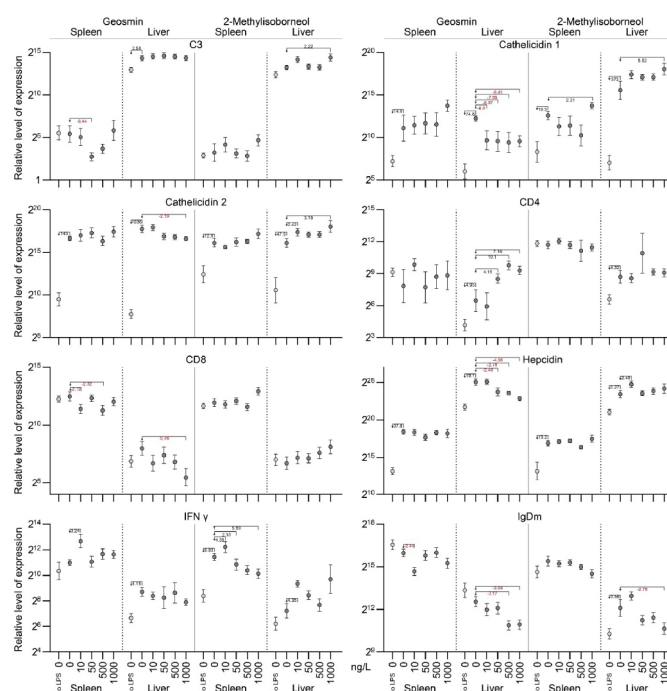
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The terpenoid compounds geosmin (GSM) and 2-methylisoborneol (MIB), produced by a wide range of microorganisms, are abundant in many aquatic environments. Both compounds are easily detectable by humans (<5 ng/L), and they are often associated with an earthy and musty unpleasant odor in drinking water and aquatic food products (such as fish and shellfish, blue food), which are costly to circumvent. These compounds are well known to bioaccumulate in larger organisms, such as fish, but the interactions between immune responses and these off-flavor compounds are not well known. We evaluated if prior exposure to these compounds can affect immune gene expression in trout.

Juvenile (~9 g) rainbow trout (*Oncorhynchus mykiss*) were exposed to geosmin and MIB in environmentally relatable concentrations as well as a high concentration (10, 50, 500, 1000 ng/L) for 24 h before IP injection of lipopolysaccharide (LPS) eliciting inflammatory reactions in trout. Following another 24 h fish were euthanized and liver and spleen were sampled for gene expression measurements (qPCR).

Both geosmin and MIB significantly altered expression of several immune-related genes in a tissue- and concentration dependent manner. Geosmin exposure was associated with a dosage dependent down-regulation in the liver of genes encoding cytokines (IL-4/13a, IL-12, TNF α), acute phase proteins (SAA, precerebellin), antimicrobial peptides (cathelicidin 1 and 2), T cell markers (TCR, CD 8), and B cell marker (IgDm). This may have implications for fish in general, but is particularly notable for those living in eutrophicated waters.

Figure 1: Dose-dependent relative expression of immune-related genes in rainbow trout after GSM and MIB pre-exposure and subsequent LPS challenge.



COMPOSITION AND BIOACTIVITY OF LIPID EXTRACTS FROM *Rugulopteryx okamurae*

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Introduction

Rugulopteryx okamurae is a species of brown macroalgae native to the Pacific Ocean that has become highly invasive along the southwestern coasts of Europe. Its proliferation has led to significant economic impacts on the fishing sector, as well as serious ecological consequences, threatening the biodiversity of marine ecosystems. To mitigate the effects associated with the accumulation of algal biomass, current research is exploring its valorization through applications such as compost production, the development of bioplastic materials, and its use as a source of bioactive compounds (Barcellos *et al.*, 2023). However, information on the functionality of bioactive compounds derived from this macroalgal species remains limited. This study aimed to characterize two lipophilic extracts obtained from *R. okamurae* using ethanol at two different concentrations and to evaluate their antioxidant and immunomodulatory properties. For this purpose, lipid extracts ROLT100 and ROLT50 were obtained using ethanol and ethanol:water (1:1, v/v), respectively. Lipid composition as well as *in vitro* antioxidant and immunomodulatory activities were subsequently assessed.

Material and methods

The brown seaweed *R. okamurae* was collected in spring from Faro de Punta Carnero (Algeciras). Algal biomass was washed, lyophilized and milled. Thereafter, 5 g of algal biomass powder were incubated in 50 ml of two different solvents: ethanol and an ethanol:water mixture (1:1, v/v), yielding a more hydrophobic (ROLT100) and a more hydrophilic (ROLT50) fraction, respectively. At the end of the extraction, the suspensions were filtered with a strainer and lyophilized. Lipid content was determined as described previously by Córdoba-Granados *et al.* 2024. Macrophages isolation was performed as described by Ponce *et al.* 2024 from head kidney of *Dicentrarchus labrax* adults (average weight 369.23 ± 21.89 g) provided by Cupibar S.L. (Cádiz, Spain). After 24h of incubation with the extracts (5, 50 and $100 \mu\text{g ml}^{-1}$) cells were collected, cell viability was determined by trypan blue assay, total RNA was isolated using the RNeasy® Mini Kit (Qiagen) and expression analyses were performed by RT-qPCR. The selected genes related to the immune system were lysozyme g (*lyg*), interleukin 1b (*il1b*), interleukin 6 (*il6*), interleukin 10 (*il10*) and tumor necrosis factor alpha (*tnfa*).

Results and discussion

The yield of the lyophilized ROLT100 and ROLT50 extracts obtained from *R. okamurae* was 13.48 ± 0.09 and $17.82 \pm 0.26\%$ of the algal dry weight, respectively. The total lipid as well as the neutral and glycolipid content were higher in the ROLT100 extract than in the ROLT 50 extract (Table 1). In the same way, ROLT100 showed a greater total phenolic content and enhanced antioxidant activity, as evidenced by higher DPPH and ABTS radical scavenging activities. Cell viability decreased significantly when macrophages were incubated with both extracts at concentrations of 50 and $100 \mu\text{g ml}^{-1}$ with respect to the controls. Therefore, subsequent gene expression analyses were conducted in macrophages incubated with ROLT100 and ROLT50 at a non-cytotoxic concentration of $5 \mu\text{g ml}^{-1}$. A significant coordinated decrease in the mRNA levels of *lyg*, *il1b*, *il6*, *il10* and *tnfa* was observed in macrophages treated with ROLT100 compared to the controls. In contrast, macrophages incubated with ROLT50 showed a significant downregulation of *lyg*, while the expression levels of *il10* and *tnfa* were significantly upregulated relative to the controls. The differential gene expression patterns observed may be partially related to the presence of diterpenoids, including dilkamural-type compounds, previously described in *R. okamurae*, which have been reported to display anti-inflammatory activities in macrophages (Cuevas *et al.*, 2021). The coordinated decreased in cytokine-related gene expression observed in macrophages treated with ROLT100 is consistent with a higher content of terpenes. Overall, the results indicate that both lipid extracts have antioxidant activity and can modulate macrophage immune responses, although the cytotoxic effects observed at higher concentrations highlight the importance of dose selection when considering their potential bioactive applications.

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Table 1
Lipid content (mg g⁻¹ dry weight of the extract) in the extracts obtained from *R. okamurae*. Values are expressed as average and standard deviation (n = 3).

	Total lipids	Neutral fraction			Glycolipid fraction		
		Fucoxantine	Fucosterol	Terpenes	SQDG	DGDG	MGDG
ROLT100	874.56 ± 18.14	136.55 ± 2.09	77.93 ± 2.07	165.52 ± 9.23	33.93 ± 1.78	20.52 ± 3.81	62.34 ± 1.68
ROLT50	427.25 ± 30.31	70.39 ± 7.46	28.04 ± 3.85	110.46 ± 17.00	30.19 ± 5.74	14.24 ± 1.68	28.61 ± 2.49

SQDG: sulfoquinovosyldiacylglycerols

DGDG: digalactosyldiacylglycerol

MGDG: monogalactosyldiacylglycerol;

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FATTY ACID DYNAMICS AND LONG-CHAIN POLYUNSATURATED FATTY ACIDS METABOLISM IN *Palaemon macrodactylus*

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Introduction

Aquaculture has grown at a rapid pace due to its key roll in satisfying the nutritional requirements for human population (Ribes Navarro, 2024). One of the major sustainability challenges is the dependence on fishmeal and fish oil coming from fisheries, and provide essential long-chain polyunsaturated fatty acids (LC-PUFAs) for many aquatic animals (Xie et al., 2021). Salt marshes and estuaries could serve as natural alternative sources of these fatty acids while preserving multiple ecosystem services (Cabral et al., 2025). *Palaemon macrodactylus* is an invasive species found in salt marshes and estuaries, highly abundant in the Guadalquivir marshes in the southwest of Spain, making it a natural resource of the area (Cuesta et al., 2006). LC-PUFAs, specifically eicosapentanoic (20:5n-3, EPA) arachidonic (20:4n-6, ARA) and docosahexanoic (22:6n-3, DHA) acids can be synthesized from short-chain polyunsaturated fatty acid precursors like linoleic acid (18:2n-6, LA) and α -linolenic acid (18:3n-3, ALA) through desaturation and elongation (Ribes Navarro, 2024). These reactions depend on two families of enzymes: fatty acyl desaturases, and elongation of very long-chain fatty acid proteins, encoded by the *elovl* and *fads* genes. Their presence, and expression have been used to evaluate the LC-PUFAs biosynthesis in aquatic organisms (Xie et al., 2021). The main objective of this experiment was to evaluate the capacity of *P. macrodactylus* to biosynthesize LC-PUFAs under controlled conditions, by examining the changes in the fatty acid profiles of two tissues and the expression of *elovl* and *fads* genes.

Materials and Methods

Samples of *P. macrodactylus* shrimp were collected from an estuary area of the Guadalquivir River to evaluate their metabolic response to diets with different fishmeal and fish oil inclusion and exploring their potential involvement in LC-PUFAs biosynthesis. Three treatments with 0%, 7%, and 14% content of fishmeal and fish oil (FM/FO) were fed for two weeks. Lipid extraction and the fatty acid composition was determined as described by Folch et al. (1957), and was analyzed in muscle and head tissues before the trial (D0), after 7 days (D7), and at the end of the experiment (D14). Relative expression of the genes *elovl4* and *fads4* was also analyzed in muscle at the same sample points, as described previously by Ponce et al. (2020).

Results and discussion

All the treatments enabled survival and growth and suggest that the diets were accepted during the two-week trial. Tissue fatty acid profiles mirrored the dietary gradient among treatments, but head reacted faster to changes, while muscle was more stable throughout the experiment. Short-chain precursors, especially LA and ALA, were higher in the head, while EPA and DHA predominated in the muscle. Expression of *elovl4* and *fads4* was highest with the FM/FO 0% treatment at D7, but differences among diets decreased by D14. Gene expression was the earliest response, followed by slower remodeling of the tissue fatty acid profile. This indicates that lipid metabolism responds to LC-PUFA availability, and the quick transcriptional response followed by the slower tissue remodeling suggests a coordinated metabolic adaptation to dietary changes (Chen et al., 2015; Feng et al., 2021). These findings confirms *P. macrodactylus*' adaptation to dynamic coastal environments (Monroig & Kabeya, 2018). Although the endogenous biosynthesis of new LC-PUFA could not be confirmed, the results provide strong evidence for the activation of the LC-PUFA's metabolic pathways and establish *P. macrodactylus* as a possible source of these fatty acids for a more sustainable production of aquafeeds.

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ANTIBACTERIAL ACTIVITY OF LIPID EXTRACTS FROM *Rugulopteryx okamurae* AGAINST *Photobacterium damsela* subsp. *piscicida*

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Introduction

Rugulopteryx okamurae is an invasive brown macroalga native to the Pacific Ocean that has rapidly expanded along the southwestern coasts of Europe, generating large accumulations of biomass and causing ecological and socioeconomic impacts. Considerable efforts are currently being made to develop valorisation strategies for this invasive resource, including its use in agriculture, biomaterials and bioproduct development (Barcellos et al., 2023). However, the biological activities of many of its metabolites remains poorly characterized. At the same time, there is growing interest in identifying natural compounds with antibacterial properties for aquaculture applications. Among bacterial diseases affecting marine aquaculture, photobacteriosis caused by *Photobacterium damsela* subsp. *piscicida* continues to impose significant economic losses in several commercially important fish species (Teixeira et al., 2023). Therefore, the aim of this study was to evaluate the antibacterial activity of two lipid-rich extracts obtained from *R. okamurae* using ethanol (ROLT100) and ethanol:water (1:1, v/v) (ROLT50) against *P. damsela* subsp. *piscicida*

Material and methods

Rugulopteryx okamurae was collected from Faro de Punta Carnero (Algeciras, Spain), washed, freeze-dried and milled. Algal powder (5g) was incubated in 50 ml of ethanol (ROLT100) or ethanol:water (1:1, v/v; ROLT50), yielding extracts with different polarity. Antibacterial activity against *P. damsela* subsp. *piscicida* was evaluated using a turbidimetric microdilution assay as previously described by Ponce et al. (2024). Bacterial suspensions were adjusted to 10^5 CFU ml⁻¹ and incubated with the extracts for 19 h at 22°C. Growth inhibition was determined from absorbance measurements at 600 nm.

Results and discussion

Total lipid content was higher in ROLT100 (874.56 ± 18.14 mg g⁻¹ dry extract) than in ROLT50 (427.23 ± 30.31 mg g⁻¹ dry extract). Despite this difference, both *R. okamurae* lipid extracts exhibited strong antibacterial activity against *P. damsela* subsp. *piscicida* after 19 h of incubation, with inhibition increasing significantly with concentration (two-way ANOVA, $p < 0.05$). ROLT50 showed significantly stronger activity than ROLT100, reaching inhibition values of 92.5% and 98.0% at 6.4 and 12.8 mg·mL⁻¹, respectively, whereas ROLT100 achieved 78.3% and 85.8% inhibition at the same concentrations. The high antibacterial activity observed for both extracts suggests that lipophilic compounds present in *R. okamurae*, including fatty acids and other bioactive lipids, may contribute to the inhibition of *P. damsela* subsp. *piscicida*. Interestingly, the stronger antibacterial activity of ROLT50 despite its lower total lipid content indicates that bioactivity depends not only on lipid quantity but also on extract composition. This enhanced activity may be associated with a higher abundance of phlorotannins and sulfated polysaccharides such as fucoidans, both of which are known to exhibit antimicrobial properties in brown macroalgae (Tang et al., 2020; Ayrapetyan et al., 2021). Nevertheless, as these compounds were not quantified in the present study, further chemical characterization and bioassays with purified fractions are required to identify the compounds responsible for the observed antibacterial activity.

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AUTOMATING SEABASS HEALTH MONITORING: A BIOLOGICALLY CONSTRAINED DEEP LEARNING APPROACH FOR JOINT SYMPTOM AND PATHOGEN DETECTION IN *Dicentrarchus labrax*

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Introduction

Pathogenic bacterial outbreaks, particularly from genera such as *Vibrio*, *Photobacterium*, and *Tenacibaculum*, pose severe threats to European seabass (*Dicentrarchus labrax*) mariculture (Lagadec et al., 2021). Mitigating these outbreaks requires continuous, objective health monitoring. Traditionally, this relies on manual recording of visual Operational Welfare Indicators (OWIs) or physical symptoms (Sauphar et al., 2026). However, manual logging is labor-intensive, highly subjective, and limited to small, infrequent samples, creating a severe bottleneck that delays veterinary intervention (Lindberg et al., 2023). While precision aquaculture started leveraging deep learning (DL) for automated visual monitoring, current models are predominantly “symptom-unaware”. They attempt to map a raw image directly to pathogen categories without identifying the specific physical indicators that justify the diagnosis. This direct mapping is highly challenging under data-scarce conditions and lacks clinical transparency required for decision-support. To bridge this gap, we introduce a novel biologically constrained Multi-Task Learning (MTL) framework that jointly predicts physical symptoms and underlying pathogens from raw images.

Materials and Methods

We evaluate this framework on CO-BASS (Co-occurring Bacterial Symptoms in Seabass), a novel expert-curated dataset of 320 standardized photographs of symptomatic European seabass. All biological annotations were performed by veterinary pathologists, establishing multi-label ground-truth categories for 8 physical symptoms (Hemorrhages, Emaciated, Skin Lesions, Abnormal Coloration, Exophthalmos, Ulcers, Damaged Fins, and Swollen Abdomen) and 5 bacterial pathogens (*Vibrio*, *Tenacibaculum*, *Photobacterium*, *Aeromonas*, and *Lactococcus*).

As illustrated in Figure 1, our proposed S2D-MTL (Symptom-to-Disease) architecture processes raw input through a shared Image Encoder branching into a Symptom Predictor and a Disease Predictor. Rather than treating these as independent tasks, a dedicated S2D Layer explicitly routes the output of the Symptom Predictor into the Disease Predictor, structurally forcing pathogen predictions to be grounded in predicted symptoms. For the S2D-MTL-Matrix variant, a co-occurrence Matrix is pre-computed from single-infection training samples. This matrix acts as a biological filter during training, penalizing the S2D Layer for learning biologically implausible symptom-pathogen connections.

Results

Our architectures were evaluated against zero-shot foundation models (e.g., BioCLIP) and standard single-task classifiers (e.g., Swin), outperforming them in both disease and symptom classification. While standard MTL improved disease classification at the expense of symptom detection (negative transfer), the structurally-constrained S2D-MTL framework reached the highest performance in disease prediction (0.94 F1 score, up to a +4% improvement over standard single-task baselines). Furthermore, the S2D-MTL-Matrix successfully improved symptom detection accuracy, reaching 0.71 F1 score and demonstrated robust performance on complex, overlapping co-infections, which are present in 62% of the CO-BASS dataset.

The dataset and code are available at <https://github.com/beatrice-portelli/CO-BASS>.

Discussion

Integrating clinical veterinary logic directly into DL architectures is a powerful strategy to overcome data scarcity in precision aquaculture. Structuring predictions to flow from physical OWIs to pathogens creates highly accurate, explainable models. For commercial sea farms, this technology paves the way for continuous, non-invasive health screening. A smart camera system running such a model could monitor fish in real-time, alerting farm managers to possible pathogen presence, while explicitly pointing out the visual symptoms (e.g., hemorrhages or damaged fins). Future work will assess model generalizability across other farmed marine species and diverse geographical regions.

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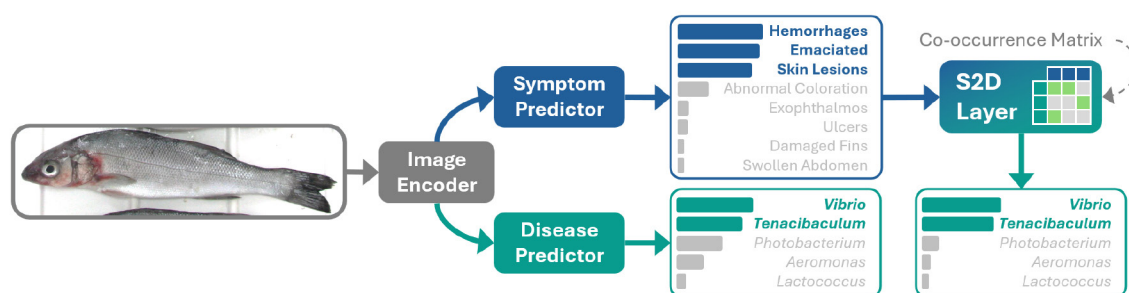


Figure 1. Diagram of the S2D-MTL model, including the co-occurrence Matrix used by S2D-MTL-Matrix variant.

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FOOD SAFETY DATA SHARING PLATFORM: BUILDING A GLOBAL INTELLIGENCE NETWORK FOR AQUACULTURE DRUG RESIDUE MONITORING

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Background

Aquaculture is among the fastest-growing sectors of the global food supply, yet managing animal drug residues in farmed seafood products remains a critical public health and regulatory challenge. Historically, residue surveillance data has been fragmented across industry, academic institutions, and government agencies, limiting the collective ability to detect emerging threats and allocate testing resources efficiently. To address this challenge, the U.S. Food and Drug Administration (FDA) developed the Food Safety Data Analytics Sharing Hub (DASHboard), managed by an independent data trustee, Creme Global. The primary objective of this initiative is to aggregate multi-partner data into a centralized, secure repository to generate collaborative intelligence that no single organization could produce alone.

Platform Overview

The DASHboard currently hosts twelve interactive visualization modules spanning a broad range of food safety hazards, including per- and polyfluoroalkyl substances (PFAS), toxic elements, microbiological hazards, marine biotoxins, and aquaculture drug residues. The Aquaculture Drug Residue (ADR) Dashboard, presented here as a key feature of the platform, encompasses more than 5,700 samples across 55 countries and 42 distinct drug residue classes. It delivers interactive visualizations and predictive analytics designed to address critical queries, such as product-country combinations with the highest risks, prevalent drug classes, and drug use patterns and trends. In 2026, the DASHboard evolved from a data repository into an active decision-support system. The DASHboard is now using a Generative AI (GenAI) toolbox to streamline food safety surveillance. This transition enables stakeholders to move rapidly from data exploration to interpretation and actionable insight. In this collaborative network, every new dataset contributed strengthens the platform's predictive accuracy, creating a compounding return on participation for all stakeholders.

Mutual Value and Impact

The platform's value proposition is straightforward: the more data contributed, the stronger the insights generated for every participant. Contributing partners, across the following stakeholder communities, gain direct access to aggregated global intelligence and can benefit from the platform's GenAI capabilities and predictive analytics:

- Aquaculture industry stakeholders can align with global trends, prevent costly violations through early residue detection, and optimize internal testing resources while establishing themselves as proactive food safety partners.
- National regulatory authorities can benefit from shared intelligence to optimize resources, prioritize testing allocations, target capacity-building and training support, and detect cross-border food safety threats earlier.
- Academic researchers can gain access to a uniquely rich, multi-source dataset to conduct large-scale collaborative studies, linking academic work to real-world surveillance and joint publication opportunities.

Data protection and the safeguarding of sensitive information are ensured through anonymization, aggregation, and the oversight of an independent trustee.

Conclusion

To advance global aquaculture safety, the FDA Food Safety DASHboard is actively expanding its data foundation through international partnerships. We invite aquaculture producers, industry associations, academic institutions, and national regulatory authorities to join our growing network. Data sharing is not a regulatory obligation; it is a strategic advantage. By participating, contributors gain exclusive access not only to a large collective dataset, but also to our newly operational GenAI toolbox, which delivers AI-powered predictive analytics, automated insights, and plain-language data exploration capabilities. The platform enables the participants to move from reactive investigation to proactive surveillance. We look forward to connecting with Aquaculture EU 2026 attendees to discuss how shared data can generate shared benefits across our industry, research, and regulatory communities.

UPWELLING ACADEMY: BUILDING SKILLS FOR INNOVATION IN AQUACULTURE AND BLUE ECONOMY

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INTRODUCTION

The blue economy is a strategic driver for the sustainable development of Atlantic regions but faces challenges such as shortages of specialised skills, loss of knowledge during early-stage innovation, and fragmentation of collaboration networks. The UPWELLING project, co-funded by the Interreg Atlantic Area Programme, addresses these barriers through an integrated approach to foster sustainable blue growth. The project aims to: (1) establish a transnational network of marine infrastructures and blue economy stakeholders; (2) design and launch the UPWELLING Academy for specialised training in marine technologies; (3) promote hackathons and proof-of-concept pilots to bridge academia and industry; and (4) enhance knowledge transfer and cooperation across regions and sectors.

Within this framework, the present work describes the design and implementation of the UPWELLING Academy and presents initial indicators of participant uptake and profile. Particular emphasis is placed on its potential as a transnational capacity-building platform for aquaculture, while integrating the sector within the broader technological and innovation context of the Blue Economy.

METHODOLOGY

The UPWELLING Academy was officially launched on 6 July 2026 and offers a two-level learning pathway. Level 1 comprises two foundational modules, *Innovation and Knowledge Transfer* and *Marine Research Infrastructures*, while Level 2 comprises four thematic modules addressing *Aquaculture and Fisheries*, *Blue Tech and Ocean Observation*, *Blue Renewable Energies*, and *Biodiversity and Resilient Coasts*.

The Academy was developed collaboratively by the UPWELLING consortium through a process combining pedagogical design, scientific and technical content development, standardisation of educational materials, Moodle-based platform implementation, and functional testing prior to launch. S2AquaCoLab coordinated the pedagogical design and development of the training programme, with contributions from IPMA and the other project partners to content development and platform implementation.

Of particular relevance to the aquaculture sector, the *Aquaculture and Fisheries* module addresses infrastructure-based monitoring, monitoring systems and sensors, data integration and key performance indicators, and precision aquaculture and artificial intelligence.

The courses are 100% online and free of charge, allowing participants to progress through the learning pathway at their own pace. Upon successful completion of each module and its corresponding assessment, participants receive a Certificate of Completion and a Digital Badge recognising the skills acquired.

Participant profile and training-interest data collected through the Academy registration process were analysed to characterise the initial audience and identify patterns of interest across the different thematic areas.

RESULTS

At the time of analysis, the participant tracker included 105 participants. The Academy attracted a diverse audience, including students (32.4%), researchers (17.1%), industry professionals (16.2%) and entrepreneurs (8.6%), as well as participants from academia, NGOs and public authorities. Overall, 90.5% of participants reported holding a Bachelor, Master or PhD degree.

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Interest was distributed across the different thematic areas of the Academy. *Aquaculture and Fisheries* was selected as the main area of interest by 7.6% of participants, while 44.8% selected all training areas. Overall, 52.4% of participants therefore included aquaculture among their expressed training interests, either specifically or as part of a broader interest in all Academy topics.

These initial data indicate interest in multidisciplinary learning pathways connecting aquaculture with innovation, digital technologies, marine research infrastructures, renewable energy and environmental resilience.

DISCUSSION

The UPWELLING Academy provides a common digital learning environment bringing together expertise from different Atlantic regions and disciplines. Its two-level structure supports both the development of transversal innovation skills and more specialised learning in areas directly relevant to sustainable aquaculture and the wider Blue Economy.

The initial participant profile suggests that the Academy is reaching both future and current Blue Economy professionals and that aquaculture-related training can benefit from being embedded within a broader multidisciplinary learning pathway. Linking aquaculture with digital technologies, research infrastructures, innovation and environmental resilience may support the development of transversal skills increasingly relevant to the sector.

As the Academy is still at an early stage of implementation, future monitoring will include participant progression, module completion, assessment performance and feedback, allowing a more comprehensive evaluation of learning outcomes and user experience. Beyond the lifetime of the UPWELLING project, the Academy is intended to remain available as a training and knowledge-transfer resource, with opportunities for additional content and expansion of its partner and user network.

Acknowledgment

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FISHEUTRUST AND THE AQUATRUST IPMA LIVING LAB: OPENING A WINDOW TO THE FUTURE OF AQUACULTURE, MEETING STAKEHOLDERS AND SOCIETY, AND STRIVING FOR INNOVATION

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Within the framework of the European project FishEUTrust, the concept of Co-Creation Living Lab (CLL) was implemented and fostered by IPMA, taking into account Portuguese contextual framework and the challenges facing aquaculture in Portugal and Europe. A CLL is a user-centered, open innovation ecosystem operating in a real-world environment where stakeholders jointly design, test, and refine solutions. In the case of IPMA, its CLL, called AQUATRUST, was conceived with the purpose to address and deconstruct the misconceptions and myths plaguing the aquaculture sector and its products, not only in the case of Portuguese consumers, but also worldwide. Although Portugal has a strong maritime tradition and high levels of seafood consumption, consumer knowledge on the origins of seafood is still limited. Hence, even though Portuguese aquaculture of fish and bivalves is growing, consumer distrust still limits its acceptance. Precisely, CLL AQUATRUST as well as the whole project FishEUTrust project aimed to strengthen consumer confidence in aquaculture products, encourage their consumption, and ease pressure on overexploited wild species. This was done by focusing on enhancing transparency, authenticity, and safety across Europe's fish supply chain. By combining innovative technologies and stakeholder engagement, it sought to augment consumer confidence in fish products. The CLL AQUATRUST has been operating by bringing together producers, researchers, consumers, and policymakers, thus generating collaborative spaces where ideas, technologies, and local knowledge converge. This participatory approach does not only enhance trust and transparency, but also leads to more sustainable and context specific solutions for the aquaculture sector. Within the FishEUTrust project, AQUATRUST and the other CLLs have served as catalysts for innovation, thereby supporting environmental sustainability, food security, and the well-being of local communities. The project was brought to successful conclusion this year, but the CLL AQUATRUST will live on with its own business model and specific concept. It strives to put innovation at the service of the sector and society by a wide range of activities: (i) close cooperation with the sector stakeholders, listening to their challenges and needs; (ii) development of joint projects/studies with sector's companies; (iii) set up of public awareness initiatives; (iv) investment in sustainability with environmentally-concerned partners, also encompassing RAS and IMTA systems; and (v) dissemination and dialogue with the scientific community. The proposed presentation is part of this dissemination purpose, showing a window to the co-created future of aquaculture, engaging stakeholders and society at large and fostering innovation.

Acknowledgment

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INTEGRATED MULTITROPHIC AQUACULTURE SYSTEMS: IMPACT ON THE CUTANEOUS MUCUS BACTERIAL MICROBIOTA OF MEAGRE (*Argyrosomus regius*)

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INTRODUCTION

Bacterial colonisation of fish external and internal surfaces, including the mucus, skin, gills, and gastrointestinal tract, is influenced by the animal's health status and the characteristics of the rearing environment. In this context, the composition and diversity of microorganisms present in water and feed may directly influence the microbiota associated with fish.

Integrated Multi-Trophic Aquaculture (IMTA) systems can alter water quality and environmental microbial communities, potentially modifying the composition of the cutaneous mucus microbiota and consequently influencing fish health and immune responses.

The aim of this study was to characterise the cutaneous mucus bacterial community of meagre (*Argyrosomus regius*) reared in different IMTA systems and assess its potential as an indicator of the microbiological conditions of the rearing environment.

METHODOLOGY

The study was conducted over seven months (May–November) at the Olhão Pilot Fish Farming Station (EPPO/IPMA), located within the Ria Formosa Natural Park. Six earthen ponds (750 m³; 1.5 m depth) were assigned to three IMTA systems, with two replicates per system, integrating meagre (*Argyrosomus regius*), white seabream (*Diplodus sargus*), and grey mullet (*Mugil cephalus*), combined with different configurations of oysters (*Crassostrea* spp.) and macroalgae (*Ulva* spp.) (Table 1). Key physicochemical water parameters were monitored daily at 09:00 h.

Six meagre were sampled monthly from each pond by rod fishing and maintained in water from their respective ponds until mucus collection to minimise changes in the cutaneous microbiota. Mucus samples were pooled by treatment, serially diluted, and cultured on TSA, TCBS, and PseudomonasCN media to assess total bacterial abundance and isolate bacteria belonging to the *Vibrionaceae* family and *Pseudomonas* genus. Plates were incubated at 23 °C, and colonies counted after 48 h and 7 days. Representative colonies were subsequently isolated, purified, and characterised using biochemical tests. Bacterial abundance was expressed as Log₁₀ CFU g⁻¹ of mucus, allowing comparison of the cutaneous microbiota among the different IMTA systems.

Species	Initial number	Treatment 1		Treatment 2		Treatment 3		Mean Weight (g)	Biomass (Kg)	Density (Kg/m ³)
		T1.1	T1.2	T2.1	T2.2	T3.1	T3.2			
<i>A. regius</i>	9000	1500	1500	1500	1500	1500	1500	200	300	0.4
<i>D. sargus</i>	5100	850	850	850	850	850	850	65	55	0.07

Table 1.: Initial stocking density and biomass characteristics of the organisms used in the experiment across the three experimental treatments (T1, T2, and T3).

<i>Crassostrea</i> spp.	72000	18000	18000	18000	18000	-	-	3	54	0.07
<i>Ulva</i> spp. (g)	200	50	50	-	-	50	50	50	0.05	

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RESULTS

Water quality showed similar seasonal patterns across the three IMTA systems. Temperature ranged from 17 to 28 °C, pH from 8.2 to 8.6, and salinity from 33.5 to 36.5 psu. Dissolved oxygen ranged between 5.2 and 7.0 mg L⁻¹, with a tendency to decrease as temperature increased, while turbidity showed greater variability, with higher values during summer, particularly in Treatment 3.

Microbiological characterisation of the meagre cutaneous mucus revealed the presence of total marine bacteria, bacteria from the family Vibrionaceae, and bacteria of the genus *Pseudomonas*. Total bacterial abundance ranged from 10³ to 10⁵ CFU g⁻¹, while *Vibrionaceae* ranged from 10² to 10⁵ CFU g⁻¹. Significant temporal differences were observed for both groups ($p < 0.05$).

Pseudomonas spp. abundance ranged from 10² to 10³ CFU g⁻¹, with the highest abundance recorded in August. No bacterial growth was detected during the warmer period, suggesting that seasonal environmental conditions may have influenced bacterial occurrence and abundance, potentially through changes in water temperature and other factors affecting fish-associated microbial communities.

Overall, the observed temporal variation in bacterial abundance suggests that seasonal environmental conditions, as well as differences in IMTA configuration, may influence the microbial community associated with the cutaneous mucus of meagre.

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EUROPEAN AQUACULTURE AS A PLATFORM FOR CULTIVATED SEAFOOD: REGIONAL OPPORTUNITIES AND BARRIERS

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Introduction

European aquaculture plays a key role in the transition toward sustainable food systems. Its growing technological base and environmental focus make it a potential platform for integrating cellular agriculture, particularly cultivated seafood (CSF). This study compares aquaculture businesses in Northern and Southern Europe, identifying regional opportunities and barriers to integrating CSF into existing production models.

Methodology

The analysis combined quantitative and qualitative data from interviews with 26 aquaculture companies across Europe. Companies were grouped by region and size to identify structural patterns including production systems and resources, partnerships, market orientation, financing models, innovation and investment capacity. Comparative analysis focused on key challenges and opportunities, identifying enabling conditions for integrating CSF into conventional aquaculture operations.

Results

Clear regional contrasts were identified. In the South, the sector consists mostly of small, family-run enterprises with limited financing but strong market orientation and regional identity. Main challenges include difficulty attracting specialised workers and competition with non-EU imports. In the North, aquaculture is dominated by large and medium-sized firms with strong investment capacity, advanced technology, and collaboration with research institutions. These conditions support innovation and potential for CSF integration. Main challenges include strict legislation and low public perception of aquaculture.

Conclusion

Integrating CSF into aquaculture could address current challenges by enhancing production efficiency, both in terms of time and space, and by promoting product diversification. Northern companies are best placed to pioneer integration. Northern Europe's aquaculture sector therefore provides a strong foundation for advancing CSF in a sustainable and inclusive way.

Acknowledgment

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DIETARY EFFECTS OF TOTAL FISHMEAL REPLACEMENT BY FULL-FAT AND DEFATTED BLACK SOLDIER FLY LARVA MEAL ON GROWTH AND FEED PERFORMANCE, HEMATOLOGICAL PROFILE, AND BLOOD PLASMA CHEMISTRY, AND CARCASS AND FILLET QUALITY OF NILE TILAPIA (*Oreochromis niloticus*), JUVENILES

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Introduction

Nile tilapia (*Oreochromis niloticus*) is the third most produced finfish species in the world. In Thailand, fishmeal is still used for the manufacturing of finfish feeds, including tilapia feeds. The fishmeal price is high, while its quality and quantity are inconsistent. Therefore, alternative proteins and the replacement of dietary fishmeal are required for continuous assessment in aquafeeds. Black soldier fly (*Hermetia illucens*), or BSF meal, has been considered as an alternative protein to fishmeal. The dietary BSF fed to tilapia species, such as Nile tilapia (*O. niloticus*), blue tilapia (*O. aureus*), and hybrid tilapia (*O. niloticus* x *O. mossambicus*), showed no adverse effects on their growth and feed performance (Maulu et al., 2022). However, the long-term dietary effects of total fishmeal replacement with BSF meal on long-term health and physiological responses, as well as carcass and fillet quality, have not been investigated for finfish species, including tilapia species. The present study aimed at evaluating the dietary effects of total fishmeal replacement by full-fat and defatted BSF meal on (1) the growth and feed performance, (2) the hematological profile and blood plasma chemistry, and (3) the carcass and fillet quality of tilapia.

Materials and Methods

To investigate the long-term dietary effects of the total fishmeal replacement by using the full-fat BSF and the defatted BSF meal. Three isoproteic (35% protein) and isolipidic (5% fat) diets (Control (Fishmeal), T1 (20% Full-fat BSF), and T2 (20% Defatted BSF) were formulated and fed to Nile tilapia (89.47 ± 0.16 g) for 24 weeks. At the end of the trial, the total weight and number of fish were recorded to calculate growth and feed performance parameters. Fish were selected to collect blood samples for hematological profile and blood plasma chemistry, organo-somatic indices, the textural profile of the fillets, and the chemical composition of the whole body.

Results

No significant differences were found in overall growth and feed performance between the control and the different treatments ($p \geq 0.05$). For hematological profile and blood plasma chemistry, the group fed the T1 and T2 diets showed significant reductions ($p < 0.05$) in erythrocyte count, Hb, and Hct, and an increase ($p < 0.05$) in leukocyte count. The values of triglyceride, cholesterol, and alanine transferase (ALT), aspartate transferase (AST), and alanine phosphatase (ALP) were higher ($p < 0.05$) for the group of fish fed the T1 and T2 than the control group. The findings show significant differences ($p < 0.05$) in VSI, HSI, and fillet yield between the T1 and T2 diets and the control diet. The highest value of fillet yield was observed in the control group (28.92%). The values of VSI and HSI were higher ($p < 0.05$) in fish fed the T1 (51.52 and 7.00%) and T2 (51.40 and 6.91%) diets when compared to the control (49.48 and 5.82%). No significant differences were found in the overall final whole-body chemical composition among the groups of fish fed control, T1, and T2 diets ($p \geq 0.05$). However, a statistical difference ($p < 0.05$) in the moisture, protein, and lipid content of the fish fillet was observed between the control group and the other treatments. A reduction trend in the overall values of the texture profile was observed in the group of fish fed the T1 and T2 diets ($p < 0.05$), except for the lightness of the fish fillet.

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Discussion

Replacing fishmeal with BSF did not negatively affect growth or feed efficiency in Nile tilapia (Maulu et al., 2022). Similarly, this study found no adverse effects on growth and feed performance. However, decreases in hematological parameters, such as RBC, Hb, and Hct, may indicate nutritional deficiencies, while elevated total WBC counts can suggest an inflammatory response. Higher blood triglycerides in fish fed BSF compared with those fed fishmeal may reflect specific nutrients or components, such as fatty acid composition, which can activate metabolic pathways and raise triglyceride levels. Since triglycerides are vital for energy storage, excessively high levels may signal an imbalance or health issue. ALP is important for intestinal functions, such as nutrient absorption, and can serve as a biomarker of tissue damage or metabolic changes in fish. An elevated ALP level may indicate damage to cell membranes in organs such as the liver, intestines, and kidneys. High dietary BSF levels may cause intestinal edema or congestion, thereby elevating VSI and HSI values. Excessive replacement of fishmeal with BSF leads to negative morphological changes and histological damage in the intestines of *O. niloticus* (Sangsawang et al., 2024). The relative sizes of organs, such as viscera (Viscero-somatic index; VSI) and liver (Hepato-somatic index; HSI), along with the proportion of edible flesh obtained from farmed fish after processing, serve as key indicators of effective feed utilization, which leads to more profitable and sustainable production. VSI and HSI are physical indicators used to evaluate fish health (Limbu et al., 2022). Complete replacement of fishmeal with either full-fat or defatted BSF significantly increased VSI and HSI and reduced tilapia fillet yield. The color of the fish fillet could be affected by dietary insect meal, such as BSF or mealworm. High inclusion levels of insect meals, such as BSF and mealworm, significantly decreased fillet yellowness. The color of the tilapia fillet showed increased redness (a^*), while yellowness (b^*) decreased with BSF larva meal, as observed here. Lower hardness, springiness, cohesiveness, chewiness, and resilience can result from increased proteolytic breakdown of muscle tissue. Notably, high BSF inclusion levels had a negative long-term impact on tilapia carcass and fillet quality.

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DIETARY EFFECTS OF TUNA SOUP STOCK FED TO HYBRID RED TILAPIA (*Oreochromis spp.*) JUVENILES

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Introduction

Tuna soap stock is a byproduct of refining tuna oil, which is commonly extracted during tuna processing, particularly in the fish oil industry. This reaction breaks down triglycerides into fatty acids and glycerol, yielding soap (a fatty acid salt) and phospholipids. The soap stock consists of fatty acids, phospholipids, impurities, and other substances removed from crude fish oil, and it can be used in aquafeeds. Research on dietary tuna soap stock in aquaculture feeds is scarce. The present study aimed to evaluate the dietary effects of tuna soap stock on growth performance, organ-somatic index, and whole-body chemical composition of hybrid red tilapia.

Materials and Methods

Four isoproteic (30.0%) and lipidic (5.0%) feeds were formulated with gradient dietary inclusion levels of tuna soap stock (0.0, 1.0, 2.0, and 3.0%), and the feeds were fed to hybrid red tilapia (*Oreochromis spp.*) juveniles for 60 days. At the end of the trial, fish samples were collected and analyzed for growth performance, organ-somatic index, and whole-body chemical composition.

Results and Discussion

The study found no significant differences in growth performance, organ-somatic index, and whole-body chemical composition among hybrid red tilapia fed diets with varying inclusion levels of tuna soap stock (0.0, 1.0, 2.0, and 3.0%). In addition, the 3% group showed inferior growth performance and lower fillet yield; however, growth performance and organ-somatic index values were not significantly different from those of the control group. This finding is important for minimizing waste and promoting resource recovery in the tuna processing industry. Tuna soap stock is a valuable by-product that can be used in tilapia aquafeeds and could effectively replace fish oil in the diet.

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FIRST HERITABILITY ESTIMATES FOR GROWTH-RELATED TRAITS IN BURBOT (*Lota lota* L.) USING A TAILORED MICROHAPOTYPE PANEL

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Introduction

Diversifying aquaculture species has become an essential strategy for improving the sustainability and resilience of the sector. Burbot (*Lota lota* L.), the only freshwater gadoid species, is well suited to diversify European coldwater aquaculture. It exhibits high fecundity and produces valuable white, mild-flavoured, boneless flesh, making it a promising candidate for commercial aquaculture (Matuha et al., 2024). However, emerging aquaculture species such as burbot require targeted genetic improvement, particularly for rapid growth through structured breeding programmes. To date, no systematic selective breeding programme has been established for this species. The improved microhaplotype panel developed for the American burbot population (Harris et al., 2024) provides a valuable foundation for developing a corresponding panel for the European burbot population. Because microhaplotypes are multiallelic loci with high heterozygosity, they provide greater statistical power than individual SNPs (Baetscher et al., 2018), enabling more accurate parentage assignment. This study aimed to optimise a microhaplotype panel for the European burbot population reared under intensive conditions, use it for parentage assignment, and, for the first time, estimate heritability for growth-related traits.

Materials and Methods

The experimental population was established using a partial factorial cross involving 26 females and 40 males from the broodstock maintained by a commercial fish farm Ryby-Vlček. Hatched larvae were initially reared in a pond to the advanced fry stage (one month) and subsequently transferred to an intensive rearing system, where they were weaned onto artificial feed. At 7 months of age, 1,436 fish were PIT-tagged and fin-clipped for DNA analysis (including 66 parental samples), and growth-related traits (body weight, standard length, head length, body height, and body width) were recorded, and Fulton's condition factor (FC), specific growth rate (SGR) and thermal growth coefficient (TGC) were calculated. The Genotyping-in-Thousands (GT-seq) (Campbell et al., 2015) microhaplotype panel was optimised through several rounds of sequencing (75-bp amplicons), followed by quality filtering of candidate markers. In addition, sex-specific markers were developed for the male-heterogametic XX/XY sex-determination system. Parentage assignment was performed using CERVUS v3.0. Heritability estimates were obtained using DMU software v.5.6, with sex included as a fixed effect, where applicable, and pedigree relationships derived from parentage assignments.

Results

The newly developed microhaplotype panel (Ryby Vlček Llo 143) contained 143 genetic markers, including 10 biallelic SNP markers and 130 microhaplotypes (3–7 alleles) and 3 sex-specific markers. The panel's mean allelic richness was 3.9, and the expected and observed heterozygosity was 0.656 and 0.598, respectively. The sex-specific markers achieved more than 96% concordance between phenotypic and genetically determined sex. The parentage assignment success rate was 99.4%, with only 8 individuals remaining unassigned. The mean body weight was 11.8 ± 3.15 g, with a coefficient of variation of 26.7%. Heritability estimates for growth-related traits were low to moderate, ranging from 0.12 to 0.36. The highest heritability estimates were observed for traits directly related to growth and body condition, namely body weight, FC, SGR, and TGC.

Discussion

A moderately high heritability was estimated for juvenile body weight ($h^2 = 0.36$), indicating promising potential for the genetic improvement of intensively-reared burbot through selective breeding. In the closely related species Atlantic cod (*Gadus morhua*), heritability estimates for juvenile body weight range from 0.16 to 0.52, across populations and model specifications (Gjerde et al., 2004; Tosh et al., 2010). In addition, FC, SGR, and TGC may also represent useful selection criteria in future burbot breeding programmes. Heritability estimates for economically important performance traits

should be evaluated throughout the production cycle until market size to determine the optimal stage for selection. Future research should also include population genetic analyses of farmed and wild European burbot populations, evaluation of triploid stocks for intensive aquaculture, characterization of the fatty acid and vitamin composition of burbot liver oil, and development of value-added food products from burbot flesh and liver.

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THE IMPACT OF BIOMASS ON WAVE AND CURRENT HYDRODYNAMIC FORCES ON A SEAWEED CULTIVATION NET

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Introduction

In an effort to increase food security, seaweed has emerged as a sustainable food option with multiple health benefits due to its nutritional value (Liboureau et al., 2026). Seaweed can be cultivated either on horizontal/vertical line systems or nets. The latter can yield higher biomass for *S. latissima* (Boderskov et al., 2023). Offshore seaweed cultivation offers more space compared to nearshore, where activities such as shipping, tourism, and fishing take place. However, moving from nearshore to the offshore high-energy environment increases the hydrodynamic force acting on the biomass, which raises the mooring load and design requirements (Tullberg et al., 2022). This highlights the importance of accurately predicting the hydrodynamic loading on an offshore seaweed cultivation system. To that end, a physical model test campaign has been conducted in Deltares' Atlantic Basin, a wave-current basin, to quantify the hydrodynamic load on a seaweed cultivation net subjected to wave and current loading. Additionally, the impact of biomass on the hydrodynamic loading was investigated. The test setup and measured forces will be made available as open-access datasets.

Materials and methods

Surrogate seaweed made of polyvinyl chloride (PVC) were used for the experiment instead of live seaweeds. The material was chosen based on its similarity to *S. latissima* in terms of density and flexural rigidity, the latter obtained from a cantilever test (Henry, 2014). Two identical 4.0 m x 0.8 m field scale nets were used, one kept clean and the other with those surrogates attached. The net was connected to four load cells, one installed at each corner, measuring the load along three axes (Figure 1). For the steady flow tests, four different current speeds were imposed: 0.10, 0.20, 0.30, and 0.38 m/s. For the oscillatory flow tests, twelve regular wave conditions were tested. Each test was repeated for three different orientations with respect to the incoming current/waves: 0°, 45°, and 90°.

Results and discussion

For the steady flow tests in 90° orientation (current perpendicular to the net), the presence of surrogates increased the measured forces by up to a factor of 10, depending on the surrogate configuration. When the surrogates were positioned on the downstream side of the net, they were lifted by the current, thereby creating openings in the net and reducing form drag. In this configuration, the main contribution to the total force came from skin friction drag induced by shear forces. In contrast, placing the surrogates on the upstream side created full blockage of the net, which induced a much larger force, coming from normal pressure forces and shear forces. At a current speed of 0.38 m/s, varying the surrogate configuration produced a wide range of measured forces (81.2 N to 460.2 N). In terms of net orientation, a 3-fold load reduction was observed when the clean net was aligned with the current direction. For the net with surrogates, the load reduction was up to 11-fold. The same trend applied to the regular wave tests, where the maximum forces were measured when the net was oriented perpendicular to the wave direction. The presence of biomass increased the force by a factor of 1.6 for the lowest Keulegan-Carpenter (KC) number and by a factor of 2.6 for the highest KC number. Furthermore, second- and third-order harmonics were observed in the measured wave amplitudes and were also visible in the measured forces.

Conclusion

An experimental campaign has been conducted in a wave-current basin to measure the hydrodynamic forces on a seaweed cultivation net. The experiment has shown that net orientation and the presence of biomass can lead to significant variations in the measured hydrodynamic forces. Both factors need to be accounted for in the design of offshore seaweed farms. This study provides open-access benchmark datasets to support the validation of numerical tools used in aquaculture system design.

(Continued on next page)



Figure 1. Seaweed net test setup in 90 degree orientation (wave/current perpendicular to the net)

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COMPARATIVE ASSESSMENT OF SEAWATER DESALINATION BRINES FROM SEMI-ARID INSULAR REGIONS OF THE EASTERN ATLANTIC — CANARY ISLANDS AND CABO VERDE — FOR THE CULTIVATION OF NATIVE MICROALGAE

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Introduction

In semi-arid island regions such as the Canary Islands and Cabo Verde, increasingly affected by freshwater scarcity, desalination represents a key option for freshwater supply, providing a reliable source of high-quality water under variable climatic conditions. However, seawater desalination generates large volumes of high-salinity brine, whose discharge requires adequate management, as it may alter receiving-water physicochemical properties and marine ecosystems. Within the framework of innovative and sustainable brine management strategies, microalgal cultivation represents a promising circular bioeconomy approach (Contreras-Gómez et al., 2026). Several halotolerant and halophilic microalgal species are able to grow under elevated salinity conditions, making them suitable candidates for cultivation in brine-based media while producing biomass with potential commercial value. Strains of *Dunaliella salina*, *D. viridis* and *Navicula* spp. have already been isolated in both the Canary Islands and Cabo Verde and tested under laboratory- and pilot-scale conditions using seawater desalination brine generated by local municipal plants as an alternative water source for culture medium formulation. Building on these previous findings, this study assessed the technical feasibility of using desalination brines from the Canary Islands and Cabo Verde for the cultivation of selected halotolerant and halophilic microalgae. This strategy supports a shift from direct brine disposal towards its valorisation as a resource for sustainable biomass production.

Materials and methods

Desalination brine samples were collected from: i) the brine outlet line of the *Matiota* desalination plant in São Vicente, Cabo Verde, and ii) the brine line at the Instituto Tecnológico de Canarias (ITC), receiving brine directly from the adjacent *IDAM Sureste* desalination plant in Gran Canaria, Canary Islands, Spain. Three annual samplings of the Canary Islands brine were conducted between 2023 and 2025, whereas the Cabo Verde brine was sampled once, in 2025. Samples were analysed in order to assess the physicochemical composition and microbiological quality. The analytical parameters were selected according to *ad hoc* characterisation plans specifically designed to assess the suitability of water inputs for microalgal cultivation and tested at ITC as a case study scenario under a previous project. Salinity and pH were analysed to determine their compatibility with optimal microalgal culture conditions. Ion and elemental concentrations were quantified to evaluate their potential contribution as nutrient sources for microalgal cultivation. Heavy metals and microbiological parameters relevant to food and feed safety were assessed to determine their suitability for biomass production intended for human and animal consumption. For the estimation of potential nutrient replacement and associated economic savings, the nutrient composition and reagent costs of a conventional f/2 medium for marine microalgae cultivation were used as the reference. For values below the limit of quantification (reported as <LOQ), the corresponding LOQ value for each parameter was used in the calculations, providing an estimate of the maximum potential cost savings.

Results

Both seawater desalination brines presented physicochemical profiles compatible with the cultivation of halotolerant and halophilic microalgae. Salinity was 55.2 in the Cabo Verde brine and ranged from 71 to 74.6 in the Canary Islands brine, whereas pH values were 8.1 and 7.5-7.6, respectively, falling within the optimal range for microalgal cultivation. In all samples, Na⁺ and Cl⁻ were the most abundant among the analysed ions; the Canary Islands brine generally exhibited higher ion concentrations than the Cabo Verde brine, consistent with its higher salinity. Through their content of NO₃⁻, PO₄³⁻, Si, Co, Cu, Fe, Mn, Mo and Zn, both brines showed potential to partially contribute to nutrients supply to the cultures and reduce supplementation costs, although the extent of this contribution varied markedly among compounds. Nitrate and phosphate were among the nutrients with the lowest estimated coverage of the f/2 medium requirements, particularly in the Cabo Verde brine, with coverage values of 0.05% and 1.02%, respectively. In the Canary Islands brines, the corresponding values ranged from 8.41% to < 45.69% for nitrate and from < 2.91% to < 8.72% for phosphate. Trace elements (Cd, Cr, Hg, Ni, Pb) and microbiological parameters did not raise major concerns when assessed against conservative reference values, supporting further evaluation of both brines for microalgae cultivation in food- and feed-related value chains.

(Continued on next page)

Discussion

These preliminary findings suggest that seawater desalination brines from Cabo Verde and the Canary Islands could serve as alternative saline water inputs for the formulation of culture media for halotolerant and halophilic microalgae cultivation. The ionic profiles of the brines were consistent with the characteristic ionic composition of seawater. The brine nutrients could reduce the need for external reagent inputs, which represent a relevant cost driver in microalgal production (Acién et al., 2014), resulting in potential nutrient cost savings of up to 8% and 41% in Cabo Verde and Canary Islands respectively. However, additional nitrogen and phosphorus inputs would still be required to formulate a nutritionally balanced medium capable of supporting optimal growth and biomass productivity in microalgal mass cultivation. Although final biomass quality and safety must be validated after cultivation, the trace element and microbiological profiles of the brines support their potential technical suitability as water inputs for microalgal cultivation intended for food and feed applications. These findings contribute to building a body of evidence that may inform future regulatory developments regarding the use of desalination brine as a water source for these applications. Overall, this work supports brine valorisation through microalgal biotechnology as a circular strategy for resource-efficient biomass production in water-scarce insular regions.

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MANDIBULAR PROGNATHISM IN EUROPEAN SEABASS ORIGINATES BEFORE LOWER JAW OSSIFICATION

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Introduction

Skeletal abnormalities are a major issue for reared fish, compromising welfare and product quality. Mandibular prognathism, which is characterized by the protrusion of the lower jaw, is one of the least investigated skeletal abnormalities in European seabass (*Dicentrarchus labrax*). While other jaw deformities have been attributed to unfavorable larval rearing conditions (e.g. Villeneuve et al., 2006), fish genetic background (e.g. Fragkouli et al., 2018) and nutritional factors (e.g. Cobcroft et al., 2001), the factors that contribute to the development of prognathism remain unknown. Identifying the developmental stage at which the deformity first becomes detectable is an essential step towards understanding the mechanisms associated with this phenotype. In the present study we a) examined whether mandibular prognathism of *D. labrax* is associated to malformations or to alterations of the relative size of the lower-jaw elements, and b) we studied the progress of the abnormality during fish ontogeny, in an attempt to identify the critical ontogenetic period.

Materials and Methods

The anatomical characteristics of prognathism were examined on ten random quality control samples which were taken from a commercial hatchery at 45 dph. Normal and deformed individuals were double-stained, photographed and subjected to geometric morphometric analysis (15 homologous landmarks, Figure 1A) (tpsDig2 v. 2.32; Rohlf, 2010). Between-group differences were assessed with Canonical variate analysis (CVA) in MorphoJ (v. 1.08.02; Klingenberg, 2011). Dentary and articular bones from normal and deformed individuals were excised and measured, while differences in their relative dimensions were tested by Mann–Whitney test ($p < 0.05$). To test whether the anatomical alterations of prognathism at 45 dph are stage and batch independent, six additional larval populations were sampled at 17, 31 and 45 dph, respectively. At 17 dph, Meckel's cartilage morphology was quantified using five lateral and two ventral homologous landmarks, whereas at 31 dph larvae were subjected to geometric morphometric analysis (MorphoJ v. 1.08.02; Klingenberg, 2011). Finally, the prevalence of prognathism throughout the different sampling ages was estimated.

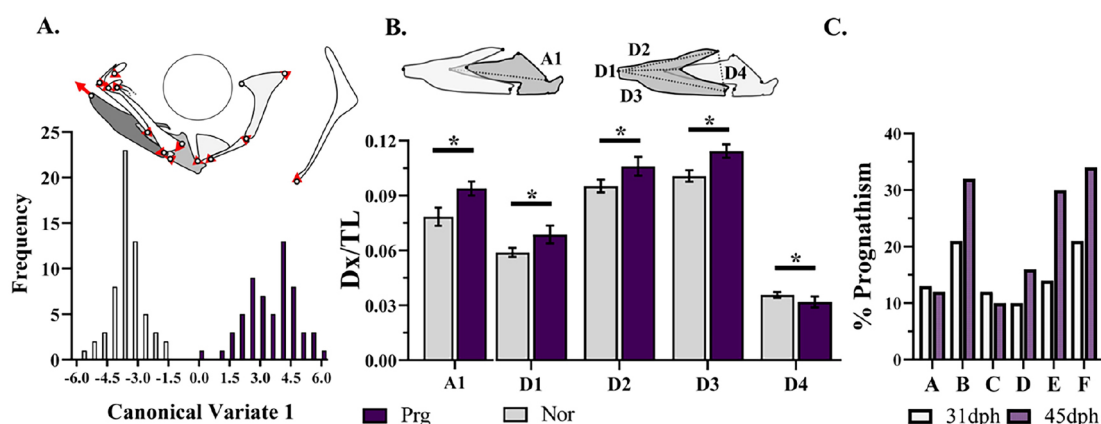


Figure 1: A. Distribution of normal (Nor) and deformed (Prg) individuals along the CV1 axis at 45 dph. Vector diagram demonstrates the comparative shape configuration of the abnormal fish. B. Mean values of the total length-standardized measurements (Dx/TL) of the dentary (D₁-D₄) and articular (A₁) bones. Asterisks (*) indicate statistically significant differences ($p < 0.05$, Mann-Witney test). Nor, normal. Prg, abnormal fish. C. Progress of prognathism % between 31 and 45 dph (second batch).

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Results

In the first batch, geometric morphometric analysis at 45 dph significantly separated normal from prognathic individuals (Pillai's trace = 0.922, $p < 0.05$). The associated shape change consisted almost exclusively of an anterior displacement of the lower-jaw tip, with no substantial alteration of the upper jaw or jaw suspensorium (Figure 1A). Separate measurements of the excised dentary–articular complex at 45 dph showed that prognathic individuals had a relatively longer dentary and articular (Figure 1B), greater dentary–articular overlap (data not shown) and reduced dentary depth (Figure 1B). This geometric morphometric pattern was confirmed in the independent second batch examined at the earlier age of 31 dph (data now shown). In the second batch, between 31 and 45 dph, prognathism prevalence remained approximately stable in two tanks and increased in the remaining four, although none of the within-tank differences was statistically significant (G-test, $p > 0.05$; Figure 1C). Finally, at 17 dph, larvae classified as potentially prognathic showed a higher Meckel's cartilage length-to-eye diameter ratio than the remaining larvae ($p < 0.05$; Figure 1D), providing suggestive evidence that the developmental divergence associated with prognathism may begin before lower-jaw ossification.

Discussion

Mandibular prognathism remains one of the least investigated skeletal abnormalities in reared European seabass, although prevalences exceeding 40% have occasionally been reported in severely affected populations (Babbucci et al., 2016). At both 31 and 45 dph, geometric morphometric analysis identified anterior displacement of the lower-jaw tip as the principal difference between normal and prognathic individuals, without substantial alterations of the upper jaw or jaw suspensorium. This displacement was associated with elongation of the dentary and articular bones. These findings suggest that prognathism primarily reflects disproportionate growth of the lower-jaw elements rather than a generalized cranial malformation. At 17 dph, potentially prognathic larvae displayed a higher Meckel's cartilage length-to-eye diameter ratio than the remaining larvae. Together with the clear phenotype at 31 dph, this suggests that prognathism is mainly established between 17 and 31 dph. From 31 to 45 dph, prevalence remained approximately stable in two tanks and increased in four, although none of these changes was statistically significant. Thus, in some populations, the deformity may continue to develop or become detectable up to 45 dph. Future studies should define the critical ontogenetic window more precisely before investigating its causative factors.

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DOES OCEAN ACIDIFICATION IMPAIR THE FUNCTIONAL PERFORMANCE OF MUSSEL BYSSUS?

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Background

Mussels attach to substrates through the byssus, a structure comprising multiple threads, rich in structural and adhesive proteins. In aquaculture, byssal attachment and integrity enable wild spat collection on farm collectors and allow adult mussels to remain secured to farming structures and neighboring individuals. Maintaining byssal performance is therefore essential for farm productivity and the climate resilience of Mediterranean mussel, *Mytilus galloprovincialis*, farming, particularly under increasingly frequent extreme weather events. Ocean acidification has been proposed to be one of the factors that impair the structural and mechanical properties of mussel byssus. Previous studies do not provide a clear pH-dependent response of individual traits [1]. Furthermore, the integrated assessment of pH effects on multiple traits remains poorly explored. This study assessed the response of *M. galloprovincialis* byssus to prolonged seawater acidification under controlled laboratory conditions. By comparing three pH levels, we evaluated the integrated byssus response, including thread production, morphometrics (length and thickness) and mechanical properties to determine whether reduced pH compromises overall byssal performance under future climate scenarios.

Materials and Methods

Adult mussels were exposed for 35 days (after a 10-day acclimation period) to three pH treatments: 8.2 (control), 7.8 and 7.4 [2,3], with three replicate tanks. In each tank, three mussels were individually placed in a Petri dish. Byssal threads were severed at the proximal end at six time points (T0-T5) over the experimental period. Repeated measurements were performed on the same individuals the day after threads were cut to assess thread production, length and diameter, breaking force, and failure location. For data analysis, observations were grouped into T0, T1 (short-term), and T2 (long-term). Trait-specific mixed-effects models appropriate for each response variable were used, with pH, time and their interaction as fixed effects and tank and individual as random effects. Model assumptions were evaluated through DHARMA diagnostics, and significant effects were followed by Tukey-adjusted comparisons of estimated marginal means. The integrated response of byssal traits was explored using principal component analysis (PCA). Statistical significance was set at $p < 0.05$.

Results and Discussion

Overall, pH did not affect byssal thread production, morphometrics, or breaking force. Temporal effects were limited and restricted to the thread length at pH 7.8. In contrast, failure location changed over time, with plaque detachment becoming increasingly frequent across all pH conditions. Independent of pH treatment, higher breaking forces were associated with a lower probability of plaque detachment. This pattern is consistent with a weakest-link response, suggesting that when the plaque-substrate interface withstands greater loading, failure may shift to the thread as the mechanically limiting component of the attachment system. Overall, byssus functional performance remained largely resistant under prolonged seawater acidification, although the temporal increase in plaque failure requires further investigation, as it could reflect repeated byssus removal, and other time-dependent processes. Multivariate analyses further supported the absence of distinct functional responses among pH treatments.

Conclusion

Under the conditions and exposure period tested, byssal functional performance was maintained across acidification treatments, suggesting that prolonged seawater acidification alone may not substantially compromise the functional integrity of the byssal attachment system of Mediterranean mussels. However, the temporal increase in plaque failures highlights the importance of considering failure location alongside conventional measures of thread production and mechanical resistance. These findings provide new insights into the functional resistance of mussel byssus under ocean acidification and may inform climate-risk assessments and adaptation strategies for Mediterranean mussel aquaculture.

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VALORIZING DUCKWEED (*Lemna minor*) TO DEVELOP A SUSTAINABLE INTEGRATED FLOW-THROUGH TROUT FARMING SYSTEM: EXPERIMENTAL SETUP AND EXPECTED OUTCOMES

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Introduction and Background

Flow-through fish farming systems are typically used to raise trout and other salmonids, which require cold and well-oxygenated freshwater, with stable flow regimes and temperatures generally below 15 °C (Bear et al., 2007). Recently, these farming systems have been studied to improve their sustainability and move toward integrated aquaculture systems with aquatic plants, such as duckweed (*Lemna minor*), that constitutes a protein-rich biomass. In addition, the integration of aquatic plants into aquaculture systems can reduce the environmental impact of fish production, primarily due to their ability to absorb nutrients and increase the efficiency of biological filtration (Paolacci et al., 2022). Duckweed is already used in lagooning basins downstream of raceways and can provide a suitable nutrient source, to be included in aquafeed (Baek et al. 2021, Fiordelmondo et al., 2022). This study will focus on the development of an integrated aquaculture pilot facility at the ‘Erede Rossi Silvio’ trout farm, located in Sefro (MC), Italy. The farm is situated in the Central Apennines area, which was severely affected by the 2016 earthquake (Bedini & Bronzini, 2018).

Methodology and Expected outcomes

Lemna minor will be harvested and collected from three different settings:

1) *Control*: ‘Natural’ collection from the lagoon basin. When the harvesting will begin, the lagoon basin will be emptied as much as possible. It will then be ‘zeroed’, and the various physical and chemical parameters of the water will be recorded. The hours of light will be calculated based on the photoperiod.

2) *Indoor tanks* (Thesis 1): An indoor laboratory close to the raceways has been built, containing two 1-m³ tanks. These tanks will collect the water pumped from the final raceway basin, each one containing bioballs. The water physicochemical parameters will be monitored, and the tanks will be fitted with 24 W, 6500 K LED lights.

3) *Outdoor tanks* (Thesis 2): Two 2-m² troughs with collected water pumped from the final basin of the raceways. The physical and chemical parameters of the water are to be monitored and the calculation of daylight hours will be carried out. No biofilter will be present.

Batches of duckweed harvested from the three theses will be weighed after draining, transported to the University of Camerino-Veterinary Medicine School (located in Matelica, MC, Italy) and dried in a laboratory oven. The duckweed will then be stored in mold-proof bags. The proximal composition, amino acid profile and heavy metal content of the samples from the three groups will be measured. Subsequently, the bags of dried duckweed will be shipped to the Cassolnovo (PV, Italy) company facility, where they will be included in three fattening experimental diets (each for the different harvesting method) at a level of 10–20% of the protein source. This will result in the production of isoprotein and isolipid diets. The theses to be compared will address different *Lemna* cultivation and harvesting methods, natural, indoor and outdoor. Trouts of 50-90g will be used in a 90-day trial.

Conclusions

This project fully adheres to the concept of sustainability as it meets its three fundamental pillars (Purvis et al., 2019): 1) environmental, as duckweed reduces the environmental impact of fish farming primarily through its nutrient absorption capacity, thus increasing the efficiency of biological filtration; 2) economic, as the plant biomass generated during water treatment can be highly productive and nutritionally valuable, potentially exploited as an alternative protein source to conventional sources, thus reducing feed production costs; 3) social, as it implements a circular economy model aimed at encouraging the innovative revitalization of aquaculture in inland areas, offering new job opportunities and opening up new avenues for several highly competitive sectors, particularly functional foods and aquaculture feed.

(Continued on next page)

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CIRCULAR NUTRITION IN *Penaeus vannamei* (BOONE, 1931): INTEGRATION OF BIOFLOC, INSECTS AND AQUACULTURE BY-PRODUCTS

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Introduction and methods

The rapid global expansion of Pacific white shrimp (*Penaeus vannamei*) aquaculture has intensified the demand for alternative protein sources to fishmeal. In this context, meals derived from *Tenebrio molitor* have already been successfully used as partial substitutes for fishmeal in diets for *P. vannamei*, with positive effects on growth, feed efficiency and immune response (Sharifinia et al., 2023). Likewise, it is believed that the larvae of this insect can be fed with biofloc residues, establishing a sustainable strategy that converts biofloc waste into protein-rich biomass. In parallel, shrimp waste and fish by-products have also been tested as viable partial substitutes for fishmeal (Kurnia et al., 2017). Therefore, the aim of this study was to test biofloc-fed *T. molitor* and aquaculture by-products as fishmeal substitute and assess their impact on the shrimps' growth.

Three containers with *T. molitor* larvae were fed for one week, being provided with wheat bran and biofloc as a dry feed source at different inclusion levels (0%, 50%, 100%) until reaching an appropriate size for producing mealworm meal. Subsequently, five isolipidic, isoproteic, and isoenergetic diets were formulated using fishmeal (FM), *T. molitor* meal (T-50 and T-100), shrimp waste (Mix1), and *Mugil cephalus* by-products (Mix2) as the main sources of animal protein.

P. vannamei specimens with an initial average weight of 1.30 ± 0.0 g were distributed in triplicate for each experimental treatment and maintained in individual 1000 L tanks within an isolated biofloc technology (BFT) system, at a density of 200 shrimp/m² for 88 days. At the end of the experiment, zootechnical parameters were calculated and 12 individuals from each treatment were sacrificed to evaluate their protein retention efficiency and intestinal microbiota through the amplification and sequencing of the 16S rRNA gene.

Table 1. Growth performance and nutritional indexes of *T. molitor* fed with biofloc at different inclusion levels.

	0%	50%	100%
Final weight (g)	0,12 ^a ± 0,001	0,10 ^b ± 0,001	0,08 ^c ± 0,001
Weight gain (g/larvae)	0,12 ^a ± 0,001	0,10 ^b ± 0,001	0,08 ^c ± 0,001
SGR (%)	1,92 ^a ± 0,498	2,85 ^b ± 0,063	3,98 ^c ± 0,159
PER (%)	2,54 ^a ± 0,016	3,99 ^b ± 0,047	8,01 ^c ± 0,012
Survival (%)	99 ± 0,1	98 ± 0,1	100 ± 0,1

Note: SGR (Specific Growth Rate); PER (Protein Efficiency Ratio)

Table 2. Growth performance and nutritional indexes obtained for *P. vannamei* fed with each experimental diet.

	FM	T50	T100	Mix 1	Mix 2
Final weight (g)	9,16 ^a ± 0,270	9,57 ^{ab} ± 0,745	11,99 ^c ± 0,165	9,52 ^{ab} ± 0,680	12,26 ^d ± 0,290
FI (% day⁻¹)	3,95 ± 0,142	3,24 ± 0,420	3,25 ± 0,159	3,81 ± 0,065	3,23 ± 0,032
SGR	2,26 ^a ± 0,047	2,31 ^{ab} ± 0,090	2,53 ^{abc} ± 0,035	2,30 ^{ab} ± 0,195	2,62 ^c ± 0,082
FCR	2,34 ± 0,070	1,89 ± 0,240	1,81 ± 0,066	2,28 ± 0,700	1,78 ± 0,012
Survival (%)	82,8 ± 8,594	79,5 ± 0,250	81,5 ± 3,156	68,8 ± 10,525	75,7 ± 7,881

Nota: DFR (Feed intake); SGR (Specific Growth Rate); FCR (Feed Conversion Rate)

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Results and discussion

Although the use of insects as a protein source for *P. vannamei* has been previously studied (Choi et al., 2018) there is a lack of research focused on recycling biofloc waste as a substrate for *T. molitor* production. In this study, the inclusion of biofloc negatively affected larval performance, showing lower final weight, weight gain, and feed conversion ratio (FCR) and protein efficiency (PER) as biofloc inclusion level increased. However, this ingredient did not affect survival (Table 1). These results may indicate that either the insects didn't consume much of the provided biofloc or that an ineffective ingredient with poorly digestibility, yet safe for larvae, with greater potential at lower inclusion levels.

As for the shrimp trial, zootechnical results indicate that the protein source influenced the growth of *P. vannamei*. The Mix 2 and T100 diets showed the highest final weights and SGR, with significant differences compared to the control (FM). Moreover, survival was high in all treatments, with no significant differences between groups (Table 2).

Therefore, these results support the feasibility of using aquaculture by-products and insect meal as total substitutes for fishmeal in shrimp diets. However, further research is needed to optimize alternative formulations in order to balance shrimp growth and feed efficiency, advancing toward more sustainable and circular aquaculture systems.

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EFFECTS OF POLYCHAETE MEAL (*Hediste diversicolor*) (MÜLLER, 1776) ON GROWTH, DIGESTIBILITY AND STRESS RESILIENCE IN *Penaeus vannamei* (BOONE, 1931)

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Introduction and methods

The current global growth of *Penaeus vannamei* aquaculture has increased the search for alternative protein sources to fishmeal. Meals derived from polychaetes (*Hediste diversicolor*) have emerged as promising candidates due to their high protein content and favorable amino acid profile for shrimp nutrition, suggesting benefits in protein digestibility and in the physiological resilience of shrimp under environmental stress conditions (Aragão et al., 2022). Additionally, preliminary studies indicate that the partial inclusion of polychaete meal in diets for *P. vannamei* maintains growth and improves certain indicators of health and feed efficiency (Heydtmann et al., 2026). Therefore, the present study aims to evaluate the effects of including polychaete meal in diets for *P. vannamei*, considering its influence on growth, nutrient digestibility, and resilience to stress.

First, three isolipidic, isoproteic, and isoenergetic diets were formulated using fishmeal (FM) and polychaete meal from *H. diversicolor* at different inclusion levels: 50% and 100% (POLI50 and POLI100). The polychaete was cultured at the IEO (Santander) and fed with organic by-products (brewer's spent grain). Subsequently, *Penaeus vannamei* individuals were distributed in triplicate for each experimental treatment and maintained in individual 90 L tanks within a RAS system, at a density of 222 shrimp/m³ per tank for 43 days. Individual growth was recorded every 15 days, and feces were collected daily. At the end of the experiment, zootechnical parameters were calculated. Last, nutritional analyses and markers in the feces (yttrium) were analyzed to determine the digestibility of the experimental treatments.

Results and discussion

The results indicate that the partial or total inclusion of polychaete meal (*Hediste diversicolor*) in diets for *Penaeus vannamei* did not significantly affect growth parameters or nutritional efficiency compared to the control diet based on fishmeal (FM). Furthermore, the final weight of the shrimp showed similar values between the control diet and the experimental treatments (Table 1), as observed in previous studies (Monteiro et al., 2024), where the replacement of fishmeal with alternative meals derived from polychaetes maintained the growth of *L. vannamei* under controlled conditions.

Likewise, polychaete meal showed an apparent digestibility coefficient (ADC %) (85.97 ± 0.680) similar to that of fishmeal (87.63 ± 1.250), in agreement with the results of (Akbaş et al., 2026), where the inclusion of marine worm meal up to 100% replacement did not compromise weight gain, digestibility, or survival.

Table 1. Growth performance and nutritional indexes of *P.vannamei* fed with the experimental protein sources.

	FM	POLI 50	POLI 100
Final weight (g)	9.53±0.835	9.15±0.824	9.56±0.827
Survival (%)	86.7±2.635	86.7 ±2.635	86.7 ±2.635
SGR (%)	4.79±0.190	4.73 ±0.187	4.83 ±0.188
FCR (feed g/ shrimp g)	1.74±0.188	1.81 ±0.188	1.72 ±0.188
DFR (% gay⁻¹)	5.81±0.488	5.95 ±0.488	5.78 ±0.488
GEC (srimp g/ feed g)	1.53±0.211	1.52 ±0.211	1.54 ±0.211

Note: SGR (Specific Growth Rate); FCR (Feed Conversion Rate); DFR (Daily Feeding Rate); GEC (Growth Efficiency Coefficient).

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No statistically significant differences were observed in survival or in feed efficiency parameters, such as the Growth Efficiency Coefficient (GEC) or the Daily Feeding Rate (DFR). This could be attributed to bioactive compounds present in *H. diversicolor*, such as peptides and natural digestive enzymes, and the good digestibility, palatability and ideal amino acid profile, which may facilitate nutrient absorption and retention. Previous studies (Aragão et al., 2022) suggest that certain polychaetes can enhance protein digestibility and enzymatic activity in the hepatopancreas of crustaceans, which could explain this trend.

Last, it is worth highlighting that the use of marine polychaetes fed with by-products to feed shrimp during the pre-growing phase represents an excellent alternative to fishmeal, as it maintains growth, feed conversion ratio, protein efficiency ratio, and survival, while also promoting circular economy practices and the sustainability of aquaculture production.

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FERMENTED INDUSTRIAL BY-PRODUCTS AS HIGH-NUTRITIONAL VALUE RAW INGREDIENTS FOR WHITE PACIFIC SHRIMP (*Penaeus vannamei*) (Boone, 1973) NUTRITON

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Introduction and methods

Nowadays, the aquaculture sector faces the challenge of finding sustainable alternatives ingredients in order to replace fishmeal as the main protein source in aquafeeds. In this context, previous studies have demonstrated that both spontaneous and bacterial-assisted fermentation of diverse raw materials can enhance their nutritional value. Notably, fermentation processes have been shown to increase crude protein content and improve amino acid profiles. Additionally, the fermentation of cereals and legumes promotes the bioconversion of complex carbohydrates, thereby increasing their digestibility (Adebo et al., 2022). Consequently, the development of sustainable fishmeal substitutes necessitates a thorough evaluation of the effects of novel protein-rich ingredients on *Penaeus vannamei* growth performance and nutrient digestibility.

To address this, various animal- and plant-derived by-products were subjected to spontaneous fermentation at 22 °C for 7 days. Crude protein content was subsequently analyzed to identify substrates exhibiting the greatest increases. Selected by-products were then further processed through three independent assisted-fermentation trials: using *Bacillus* spp., *Streptococcus* spp., and a mixed culture of both genera. Finally, the complete amino acid profile of each by-product subjected to the three independent bacterial-assisted fermentation treatments was determined. Those materials exhibiting increased levels of lysine, methionine, and arginine were identified as promising ingredients for feed formulation.

Results and discussion

The crude protein content of all tested substrates increased after fermentation, regardless of the treatment applied, confirming the general effectiveness of microbial processes in enhancing protein concentration (Table 1). Nevertheless, bacterial-assisted fermentation proved more effective than the spontaneous process across all substrates, showing consistent results with previous works (Medeiros et al., 2018).

Table 1.Crude protein expressed as dry matter percentage (CP (dm%)) of the different tested materials before and after the fermentation processes.

	CP (%) Before fermentation	CP (%) After Fermentation			
		Spontaneous	<i>Bacillus</i> spp	<i>Streptococcus</i> spp	Mix B+S
<i>Ulva lacinulata</i>	20.59±0.001	21.70±0.320	29.93±0.970	30.18 ±0.480	36.41±0.630
Soybean meal	50.37±0.001	53.81±0.620	56.67±1.550	56.64 ±0.860	56.09±1.810
Degreased cameline	38.20±0.001	40.54 ±0.230	40.90 ±1.660	41.90 ±0.860	40.90 ±1.810
Degreased canola	39.95±0.001	42.80 ±0.140	47.40 ±0.520	46.40 ±0.700	46.77 ±0.830
Flax	21.51±0.001	24.09 ±0.260	25.34 ±0.090	24.13 ±0.001	23.11 ±0.220
Sunflower seeds	20.63±0.001	21.26 ±0.630	21.71 ±1.030	19.52 ±0.550	18.46 ±1.630
Brewer's spent grain	27.94 ±0.001	29.01 ±0.210	40.38 ±1.820	40.22 ±3.060	35.64 ±9.370

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Bacillus spp. generally yielded the highest CP increases in soybean meal, degreased canola, flax sunflower seeds and brewer's spent grain, likely due to its ability to release proteases and peptide enzymes that allow a better degradation of the substrate (Liu et al., 2023). On the other hand, *Streptococcus spp.* showed substrate-specific advantages, particularly in degreased cameline. Last, the combined inoculum demonstrated a clear synergistic effect only in *Ulva laciniulata*, suggesting that microbial interactions depend strongly on substrate composition and biochemical accessibility (Chen et al., 2025).

Overall, these findings reinforce the importance of selecting appropriate microbial strains tailored to each substrate to optimize protein enrichment. Furthermore, additional research is required to assess the digestibility of the selected by-products, with the aim of formulating alternative diets and subsequently conducting in vivo growth trials using *Penaeus vannamei* specimens.

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EXPERIENCE FROM EXPOSED OFFSHORE SEAWEED CULTIVATION IN AN IMTA PILOT IN SOUTHERN PORTUGAL - CHALLENGES AND OPPORTUNITIES

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Introduction

Despite mixed market signals over the past years, it is expected that the European seaweed market will experience steep growth (Jueterbock et al., 2025, Mendes et al., 2022), and the scarcity of suitable near-shore areas will require the expansion of the matured industry towards exposed offshore cultivation. In Portugal, practically no suitable near-shore areas exist for the early phase of offshore seaweed farming, but the potential of exposed offshore sites is vast. The co-location of seaweed with established fish farms in an Integrated Multi-Trophic Aquaculture (IMTA) approach has been widely discussed over the last years (Buck et al., 2028), but in practice it has not been implemented in existing near-shore farms. Lack of short-term economic benefits and space restrictions around existing near-shore farms – in particular in Northern Europe – are the likely main reasons for this. The move towards offshore can attenuate these obstacles, allowing for promising operational synergies and shared infrastructure that the IMTA concept can bring along. Both the potential for IMTA operation in an offshore farm and the performance of native species in these highly dynamic environments in Portugal remains poorly understood. This study evaluates a two-season pilot project in the Algarve (southern Portugal), detailing the technical, logistical, and biological results, challenges and opportunities for scaling offshore cultivation of native macroalgae.

Materials and Methods

A 1-hectare offshore mooring grid was installed adjacent to existing fish cages at a depth of 25–30 m. The system was designed with 50 m transverse longlines suspended at a depth of 3 m to support the seeded substrate. Target species focus was on native kelps (*Saccharina latissima*, *Laminaria ochroleuca*), *Saccorhiza polyschides* and tentatively the chlorophytes and *Ulva* sp., *Codium* spp. and the rodophyte *Porphyra umbilicalis*. Seedlings were cultivated in onshore hatcheries, transported in controlled thermal conditions, and deployed at sea between October and March, partly over 2 seasons. Operations were intentionally adapted to rely on existing infrastructure, vessels and operational schedule of the existing finfish operations, and seaweed farm operations like deployment, monitoring were conducted with a small vessels (5–6 m) to validate the feasibility of light logistical maintenance without disrupting the primary heavy-machinery needs of the fish farm.

Results and Discussion

In particular for the larger brown seaweeds, the growth trials showed promising results, despite the short and constrained match of operation windows for hatchery and sea operations. The six macroalgae had different levels of hatchery know-how and former experience both in lab- and sea-scale, and in particular the results for the rodophyte and chlorophytes were less conclusive. The offshore environment presented significant biological and technical constraints. Heavy biofouling proved to be a primary challenge; small epiphytes and mussel encrustations rapidly colonized the substrate lines, outcompeting and suffocating the seaweed seedlings before they could establish significant biomass, particularly in the case of *Codium* spp.. Herbivory signs were also observed, most noticeably in *L. ochroleuca* blades. Mechanically, the structure sustained minor but impactful damages, such as broken seeded threads and friction wear caused by crossing ropes and hydrodynamic tension. Furthermore, logistical execution was heavily constrained by strict biological deployment windows colliding with winter weather instability and the simultaneous operational demands of the fish farm.

Despite the high biological pressure from biofouling and herbivory, this pilot demonstrates that offshore IMTA presents viable opportunities for spatial expansion and the reduction of operational costs through shared maritime logistics. Managing the narrow deployment windows requires a delicate balance between optimal seaweed transfer times and the daily priorities of fish husbandry. Overcoming the observed encrustation bottlenecks will require further optimization of deployment timing, depth adjustments, and the establishment of routine offshore maintenance techniques and protocols. Ultimately, these initial trials establish a crucial technical foundation for developing competitive, large-scale offshore macroalgae aquaculture and advancing the Blue Bioeconomy in Portugal.

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USE OF THE INVASIVE MACROALGA *Caulerpa racemosa* AS A POTENTIAL FUNCTIONAL INGREDIENT FOR GILTHEAD SEABREAM (*Sparus aurata*)

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Introduction

The use of marine macroalgae in aquaculture is gaining attention as a sustainable alternative for feed formulation, particularly in species such as gilthead seabream (*Sparus aurata*). The traditional reliance on fishmeal and fish oil poses significant economic and environmental challenges, driving the search for alternative nutrient sources, among which macroalgae stand out. Recent studies have shown that including 5% of species such as *Gracilaria gracilis* enhances immune and antioxidant status in seabream, improving growth performance and disease resistance (Passos et al., 2021). Among the most promising candidates, the green macroalga *Caulerpa racemosa*, native to Asia but widely distributed in tropical and temperate regions, is notable for its high content of bioactive compounds, including phenolics, flavonoids, fatty acids, terpenes, and alkaloids. Dietary supplementation trials in aquaculture species such as *Cirrhinus mrigala* have demonstrated that *C. racemosa* improves growth, digestive enzyme activity, and feed conversion ratio (Ragunath et al., 2024). Additionally, 240 mg/kg of its extract reduced intestinal damage in *Litopenaeus vannamei* infected with *Vibrio parahaemolyticus* (Pratiwi et al., 2022). These findings suggest that *C. racemosa* acts as a functional additive with nutritional, immunological, and environmental benefits, supporting sustainable aquaculture practices.

Materials and methods

Dried *C. racemosa* biomass was used to obtain lipophilic and aqueous extracts to evaluate antimicrobial activity against *Vibrio anguillarum*, *Vibrio alginolyticus*, and *Photobacterium damsela* using the disk diffusion method following Uddin et al. (2020). Additionally, 270 juvenile gilthead seabream (*Sparus aurata*) were randomly distributed into 15 fiberglass tanks (400 L; 18 fish per tank), with three replicates per treatment in an open seawater system under natural photoperiod. Fish were fed to apparent satiation twice daily, six days per week, using five experimental diets: a commercial control and four diets supplemented with 0.75%, 1.5%, 3%, and 6% *C. racemosa* biomass. Uneaten feed was collected, dried for 24 h, and weighed to calculate feed intake (FI) and feed conversion ratio (FCR). The trial lasted until fish doubled their initial weight, followed by final sampling for histological analysis.

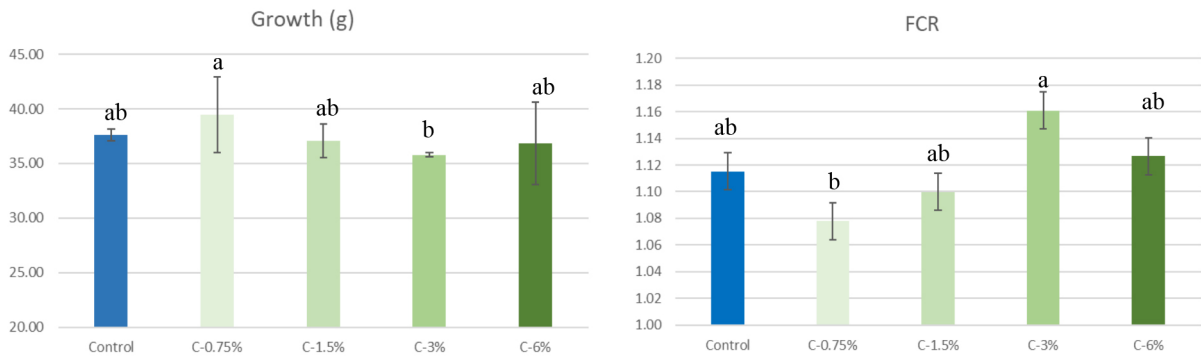


Figure 1. Growth performance and feed conversion ratio of juvenile gilthead seabream (*Sparus aurata*) fed the experimental diets. Columns with different superscripts indicate significant differences among treatments ($p < 0.05$).

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Results and discussion

No antibacterial or bacteriostatic effects were observed in the in vitro analyses, as inhibition halos were absent in all tested extracts concentrations against the studied bacterial strains. In contrast, fish fed 0.75% *C. racemosa* showed the highest final weight, significantly differing from the 3% group. A similar trend was observed for specific growth rate (SGR), although differences were not significant. Feed utilization improved at 0.75%, showing the lowest FCR and significantly outperforming the 3% group. Preliminary histology analyses revealed comparable liver steatosis, glycogen accumulation, peripancreatic fat, and hyperemia among treatments. Moreover, a tendency toward reduced steatosis (~50%) was observed in the 0.75% treatment compared to the control (not statistically significant), along with increased fold length, area and goblet cell count in the anterior gut, which suggests improved physiological functionality. Overall, all treatments performed similarly to the control, indicating that inclusion of up to 6% *C. racemosa* does not impair growth or tissue integrity in gilthead seabream (*Sparus aurata*). However, additional analyses of tissues and plasma are ongoing to better understand the full potential of this ingredient as a functional additive for sustainable aquaculture.

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EFFECT OF DIFFERENT FEEDING STRATEGIES ON JAW DEFORMITIES, STRESS AND MORTALITY IN BALLAN WRASSE *Labrus bergylta*

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Introduction

The cleaner fish Ballan wrasse have long been established as an effective measure to control salmon lice. However, the commercial production of this species is compromised by welfare related disorders such as fin erosion (biting/aggression) and jaw deformities. This eventually leads to a “poor quality” cleaner fish, resulting in high mortalities in salmon cages (National Inspection Campaign 2018/2019). The prevalence of jaw deformities has been reported to be as high as 33 %, however the cause is elusive (Cavrois-Rogacki *et al.*, 2021). Ballan wrasses undergo a distinct ontogenetic shift in feeding strategy, transitioning from zooplankton in early larval stages using pharyngeal teeth to ensure that prey doesn’t escape, to benthic foraging using maxillary and dentary teeth to ingest prey from a surface. This is associated with ossification of the skull and maturation of the dental apparatus alongside the developing digestive system (Norland *et al.*, 2022). However, in captivity, wrasse is fed with conventional pelagic feed delivery from weaning and onwards. We have therefore hypothesized that depriving the species natural foraging is disrupting normal bone development of the jaw (Ding *et al.*, 2024). This grazing feeding habit of larvae supports the hypothesis that substrate feeding is more appropriate for this species during critical ossification windows in early development, thereby reducing the deformities during production. Thus, this study aimed to investigate whether providing feed on a substrate (feed pasted on to a pipe) rather than pelagic feed (feed dispersed onto the water using a belt) could reduce biting behavior or jaw deformities, and stress thereby improving the welfare in Ballan wrasse.

Materials and Methods

The newly hatched larvae were distributed into six 500 L tanks (B5, B6, B7, B8, B9 and B10) with seawater (35.0 ± 0.5 ppt) and maintained under constant light. The larvae were start fed with rotifers from day 3 post-hatch, followed by artemia from day 25 post-hatch. Weaning started at day 52 post-hatch. Feeding substrate/pipe feeders were introduced into three tanks (B6, B8 and B10) as the treatment group and the control tanks (B5, B9 and B9) larvae were fed using an automatic belt feeder. The Experimental feeding substrates were prepared by mixing a known quantity of the dry feed (Aglonorse Berggylt Start – 0.2-0.3 mm – TROFI) with an agar-based solution until a consistent texture was achieved as previously described by Leclercq *et al.*, 2015 with slight modifications (Leclercq *et al.*, 2015). This feed mixture was then pasted onto a pipe (52 mm x 15 cm) covered with a cotton fabric for a better adherence of feed particles. The pipe was stored for 24-48 h at 5 °C prior to immersion. For each experimental treatment group, three pipes were placed during the start of the experiment and later replaced accordingly with fresh feeders based on the feed left on to the pipe. The net weight of the pipe feeders was measured before and after its deployment to the tank to record the amount of feed leached or eaten. Adjustments were made to the amount of feed in the substrate feeders to match the feed dispersed using belt feeders to keep the feed quantity consistent across these groups. Further, underwater video recordings were also performed to monitor the feed intake and behaviour of larvae/juveniles. The water temperature ranged between 12-15°C for 1-4 weeks days post hatch (dph), and 16-18°C from 5-14 weeks dph. The water flow was maintained at 50 L h⁻¹ during the start of the feeding trial and increased to 300 L h⁻¹ at the end of the trial. At the end of the experiment, larval growth and mortality were recorded. The water quality parameters such as turbidity, and microbial community were also monitored. Hormones such as cortisol, melatonin and serotonin were measured using Liquid Chromatography-Mass Spectrometry (LC-MS) to monitor the general wellbeing of larvae. At the end of the experiment, larvae/juveniles were scored manually and imaged using micro-CT for recording jaw deformities.

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Results

A high prevalence of jaw deformities was observed in the belt fed group which were characterised by short or curved upper/lower jaw. The most common deformity observed was short or askew snout. The recorded deformities were significantly higher ($P < 0.05$) in the belt fed group with 33%, than the substrate fed group with 9%. There was no significant difference in turbidity measurements among the different groups, however the substrate fed group had relatively less turbidity than the belt fed group. The microbial community in the water was not significantly ($P > 0.05$) different between the two feed treatments, and the main change in microbial community composition observed was associated with the switch from artemia to the dry feed. Hormone analysis on the whole larvae indicated no significant differences among the group. However, the cortisol levels (ng mg^{-1} of sample) corresponded with the mortality pattern in the trial, reaching peak level at 67 dph with substrate fed group (9.9 ± 5.02) had relatively higher level than belt fed group (6.4 ± 3.60). The cumulative mortality (%) were significantly higher ($P < 0.05$) in the substrate fed group than belt fed groups. This difference was also reflected in the final growth where belt fed group had a significantly higher growth (mm) (43.86 ± 7.00) than the substrate fed group (34.19 ± 7.38).

Discussion

A functional jaw is critical for delousing efficiency, and fish with jaw deformities can cause significant economic and welfare losses for hatcheries. Previous studies have reported a high percent of jaw deformities indicating production and husbandry practices as a cause for higher deformities (Cavrois-Rogacki *et al.*, 2021). This study showed lower incidences of jaw deformities in substrate fed group compared to belt fed group. This could be because, the feed presented on a solid surface mimics the grazing behaviour observed in wild ballan wrasse which is critical for their dentary ossification process. However, the current study reports a lower survival rate in substrate fed group. Such low survival rates are also reported from commercial rearing of Ballan wrasse hatcheries with rates as low as 3-5% (SINTEF Ocean, 2023). Thus, low survivability in the current study indicates a reduced feed accessibility or feed quantity or feeding efficiency in the substrate fed group during the trial. This was also reflected in the lower weight in this group compared to belt fed group. Reports have suggested that larval survival is better when they are fed continuously (Helland *et al.*, 2014). Therefore, the amount of feed introduced on to the pipe needs to be adjusted and optimised for sufficient feed intake. This reduced feed availability might have caused stress, elevating the cortisol levels, reducing the survival across the trial. Collectively, these findings suggest a trade-off between improved growth and survival under belt feeding and improved morphological welfare under substrate feeding.

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AI-DRIVEN SMART AQUACULTURE: AN INTEGRATED MONITORING, PREDICTION, AND DECISION-SUPPORT SYSTEM

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Aquaculture is increasingly dependent on continuous monitoring and timely management of environmental and production conditions. Variables such as dissolved oxygen, temperature, pH, turbidity, biomass, growth, feeding efficiency, and fish behavior are strongly interconnected, while conventional monitoring approaches often consider these factors separately. Recent advances in artificial intelligence (AI), machine learning (ML), Internet of Things (IoT), and computer vision provide an opportunity to transform aquaculture management from predominantly reactive practices toward predictive and data-driven decision support. Recent reviews have highlighted applications of AI and deep learning in water-quality forecasting, biomass estimation, fish detection and counting, growth prediction, health monitoring, feeding optimization, and behavioral analysis, while also identifying data availability, model generalization, and real-world validation as major challenges [1–3].

This work presents an AI-driven smart aquaculture architecture designed to integrate heterogeneous information sources into a unified monitoring, prediction, and farm-level decision-support system. The proposed architecture combines three complementary data streams: IoT-based environmental measurements, farm production and intervention records, and fish images/video. Instead of treating individual ML models as independent prediction tools, the architecture organizes their outputs within a common risk-fusion and decision-support layer. The objective is to convert model predictions into interpretable operational information that can support actions such as increasing aeration, adjusting feeding strategies, investigating water chemistry, inspecting fish health, and scheduling management interventions. The overall architecture is therefore intended not simply to improve prediction accuracy, but to connect prediction with actionable farm management.

System Architecture and Methodology

The ML layer is organized into five specialized analytical models, each addressing a distinct aspect of aquaculture management. The first model uses a Long Short-Term Memory (LSTM) network to forecast dissolved oxygen (DO), an important indicator of water quality and fish stress. The second is a proposed LSTM model for forecasting pH and identifying potential water-chemistry risk. The third uses XGBoost regression to estimate total fish biomass. The fourth is a dedicated gradient-boosting regression model intended to predict growth and feed-efficiency indicators. The fifth is a planned YOLO/CNN-based computer-vision model for fish detection, counting, size estimation, and behavioral or health assessment. A Vision Transformer (ViT) is also identified as a candidate architecture for the computer-vision component. The study uses the `Data_Model_IoTMLCQ_2024.xlsx` dataset [4], containing approximately 6,216 observations collected from January to June. The available variables include average fish weight, survival rate, disease occurrence, temperature, dissolved oxygen, pH, turbidity, temporal information, and intervention indicators. Data preparation includes chronological ordering, validation of temporal fields, construction of Date, Time variables, lagged features, rolling statistics, cyclic encoding of hour, feature scaling for LSTM models, and construction of sequential input windows. Production variables are additionally engineered to support biomass, growth, and feed-efficiency analysis. For the computer-vision component, the proposed system requires appropriately annotated underwater images or video frames containing labels for detection, counting, size, behavior, and health.

Implemented Models and Experimental Results

The currently implemented components provide encouraging initial results. The DO-LSTM prototype uses environmental and temporal information together with intervention records and employs a 70% training, 15% validation, and 15% testing division. Its reported performance is $MAE = 0.18$ mg/L, $RMSE = 0.27$ mg/L, and $R^2 = 0.95$, indicating strong predictive performance for the available development data. The predicted DO is subsequently transformed into a risk score, allowing anticipated low-oxygen conditions to trigger an aeration-related recommendation.

The second implemented component is the biomass-XGBoost model, which uses fish age, fish count, feed quantity, environmental variables, survival, disease occurrence, and growth-related indicators. The reported performance is $MAE = 12.4$ kg, $RMSE = 18.7$ kg, and $R^2 = 0.97$. The resulting biomass estimate can support feed planning, stock management, harvest forecasting, and production optimization, and is also converted into a biomass-risk indicator for integration with the overall decision-support layer.

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COMPUTER VISION-BASED ASSESSMENT OF OLFACTORY AND GUSTATORY RESPONSES IN RAINBOW TROUT *Oncorhynchus mykiss*

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Introduction

Palatants are often used in aquaculture to enhance feed palatability and improve feeding efficiency. However, there is a lack of methodological approaches to evaluate fish feeding responses, and conventional metrics such as feed intake may not fully capture all effects, particularly those related to pre-ingestive sensory responses. This study evaluated the olfactory and gustatory responses of rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with a classic marine hydrolysate (MH) and a non-animal-based palatability enhancer (PE), using both traditional intake measurements and computer vision-based behavioural analysis.

Materials and Methods

Three isoproteic (37%) and isolipidic (16%) diets were tested: a control (CON) and diets supplemented with either a marine hydrolysate (MH; 1.50%) or a non-animal-based palatability enhancer (PE; 0.15%). Fish were reared in a recirculating aquaculture system and subjected to two experimental phases: optimal conditions and stress conditions (hypoxia–crowding and thermal stress). Feeding behaviour was assessed through feed intake measurements and computer vision analysis using a YOLO-based model. Behavioural metrics were classified as indicators of gustatory responses (pellet ingestion dynamics and swimming activity during feeding) and olfactory responses (fish aggregation in the aroma zone and distance to the feed source prior to ingestion). Statistical analyses included ANOVA and generalized additive mixed models (Wood, 2017).

Results and Discussion

Daily feed intake did not differ significantly among diets under either optimal or stress conditions ($P > 0.05$), indicating the limited sensitivity of conventional metrics. However, behavioural analyses revealed clear diet-specific effects. The MH promoted faster pellet ingestion and increased swimming activity, suggesting enhanced immediate feeding stimulation. In contrast, the PE consistently increased fish aggregation near the feed source, reduced fish-to-feed distance, and maintained stronger attraction under stress conditions, particularly under thermal challenge.

Notably, the non-animal-based PE showed a more sustained and robust effect on olfactory-driven behaviours across conditions, supporting its role in enhancing feed detection and feeding motivation over time. These findings indicate that while MH primarily stimulates short-term ingestion responses, PE modulates broader sensory pathways, contributing to improved feed acquisition efficiency, especially under suboptimal environmental conditions. Such effects are not captured by feed intake alone but are critical for understanding complex feeding responses. Computer vision-based behavioural quantification further enabled high-resolution assessment of these responses (Bradski, 2000).

Conclusion

Computer vision-based metrics reveal that palatants can significantly influence feeding dynamics beyond conventional intake measurements. The non-animal-based PE, compared to the marine hydrolysate, demonstrated a more consistent and robust effect on fish attraction and feeding-related behaviours, particularly under stress conditions. These results highlight the importance of integrating behavioural tools to better characterize and differentiate palatant effects in aquafeeds.

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INTRINSIC OR INTERPRETED? ENVIRONMENTAL BENEFITS OF AQUACULTURE INTEGRATION DEPEND ON EVALUATION PERSPECTIVE

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Introduction

Aquaculture is increasingly expected to expand food production, with growing emphasis on circular and integrated systems. Although aquaculture generally exhibits lower environmental impacts than terrestrial livestock, sustainability cannot be inferred from output alone, as environmental burdens are unevenly distributed across systems and scales (Naylor et al., 2021; Xu et al., 2022). Integrated systems are often assumed to outperform monoculture due to resource recycling and trophic complementarities; however, such conclusions are highly sensitive to how performance is defined and measured. Life cycle assessment (LCA), the primary tool for environmental evaluation, shows that functional unit, system boundary, and allocation choices are key sources of variability, limiting comparability (Henriksson et al., 2012; Philis et al., 2019). Despite this, most studies treat integration benefits as intrinsic system properties rather than outcomes of the evaluation framework. Here, we directly test this assumption.

Methodology

We analysed harmonized life cycle inventory data comparing shrimp monoculture and shrimp–tilapia integrated aquaculture from a controlled, replicated greenhouse experiment, with both systems operated concurrently at the same location to minimise extrinsic variability. Eight model configurations combined three co-product allocation approaches (mass, economic, protein) with alternative system boundaries (including or excluding infrastructure). Environmental impacts were assessed across 18 midpoint categories using the ReCiPe method in SimaPro and re-expressed under four functional units: 1 kg total biomass, 1 kg edible biomass, 1 kg edible protein, and 1 ha·year⁻¹ land productivity. Non-parametric tests were used to evaluate whether methodological choices systematically altered apparent environmental performance.

Results

Methodological framing strongly altered the interpretation of integration benefits. The most pronounced effect arose from functional unit choice, which systematically altered comparative outcomes. When expressed per hectare-year of land productivity, the integrated system outperformed monoculture in 17 of 18 impact categories including reductions of 31.7% for freshwater eutrophication, 33.1% for marine eutrophication and 27.2% for land use. In contrast, the integrated system was favoured in only 6 of 18 categories per kilogram of total biomass, and in just 4 of 18 categories when expressed per kilogram of edible biomass. Across the eight model configurations, overall rankings differed markedly among impact categories (Friedman $\chi^2 = 84.94$, $p = 1.35 \times 10^{-15}$; Kendall's $W = 0.674$). Allocation effects were present but comparatively smaller in magnitude. Within the integrated system, mass allocation produced the lowest environmental burdens, compared to protein (1.4% increase than mass) and especially economic allocation (3.1% increase). System boundary definition exerted a consistent quantitative influence: excluding infrastructure reduced median impacts by approximately 20–22% across model configurations, indicating that infrastructure is a non-trivial contributor to total impacts.

Discussion

These results show that the environmental advantage of integration is not intrinsic or universal; it is interpreted through the analytical lens used to evaluate it. This supports previous reviews (Henriksson et al., 2015; Ruiz-Salmón et al., 2021), but goes further by demonstrating within a harmonized dataset that conclusions can reverse even when biological data remain unchanged. Our results show that functional unit selection alone can reverse sustainability conclusions, consistent with earlier observations that shifting from live-weight to edible or nutritional units alters system definition and comparability due to differences in edible yield and nutritional value (Henriksson et al., 2015). The dominance of land-based functional units indicates that integration primarily enhances spatial and multifunctional productivity, aligning with studies showing that polyculture benefits from species complementarity but not uniformly across impact categories (Bohnes et al., 2019;

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Chary et al., 2020). Allocation remains a known challenge in seafood LCA due to system multifunctionality (Ayer et al., 2007), and while its effects were smaller here, prior IMTA work confirms that allocation choices can determine comparative outcomes (Mendoza Beltran et al., 2018). System boundary effects further underscore the importance of modelling assumptions: the ~20–22% reduction in impacts when excluding infrastructure is consistent with evidence that capital goods and energy use can dominate impacts in integrated systems (Elnour et al., 2023), and that boundary definition governs which processes are attributed to the system. Overall, our results challenge universal claims of environmental superiority for integrated aquaculture. Instead, integration redistributes performance across functions, improving land-use efficiency while not consistently reducing burdens per unit of edible output. The implication is clear: sustainability conclusions depend as much on how systems are evaluated as on how they perform.

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DIETARY SUPPLEMENTATION WITH A MACRO- AND MICROALGAE BLEND MODULATED SKIN MUCUS IgM AND PROTEOMIC RESPONSES IN TURBOT (*Scophthalmus maximus*) BREEDERS

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Introduction

Improving broodstock condition is a key goal in turbot (*Scophthalmus maximus*) aquaculture, as breeder status can influence reproductive performance and consequently offspring quality. Functional diets based on sustainable marine ingredients may help support broodstock robustness, but their effects remain poorly characterized, particularly through non-lethal approaches. This study delves into turbot breeders, a valuable flatfish for human consumption, predominantly farmed in Southern European countries, Iceland, and Norway.

Skin mucus is particularly relevant in this study, not only because it allows minimally invasive repeated sampling, but also because it represents the first line of defence in fish and contains a wide range of immune effectors involved in protection against external challenges, thereby reflecting mucosal immune status. This study aimed to evaluate whether dietary supplementation with a macro- and microalgae blend modulates systemic humoral responses and skin mucus immune/proteomic profiles in turbot breeders during the reproductive season.

Materials and Methods

A four-month feeding trial was conducted at Flatlantic fish farm using approximately 200 breeders distributed across four 16 m³ tanks (2 Control and 2 ALGAE). Fish were fed either a commercial semi-moist broodstock diet (Control) or an isoproteic, isoenergetic experimental diet (ALGAE) supplemented with an algae blend (*Spirulina platensis* and *Laminaria digitata*) and micronutrients (astaxanthin, vitamin C, and vitamin E). The ALGAE diet aimed to enhance the immune status of turbot breeders during the breeding season. Minimally invasive and non-lethal sampling techniques (8 fish/tank) were performed for the collection of blood and skin mucus to study humoral parameters and proteomics in skin mucus.

Results

Plasma responses were modest. Lysozyme activity tended to be higher in fish fed the ALGAE diet, particularly in females, whereas bactericidal activity was higher in control males. In contrast, skin mucus showed a more pronounced response to dietary supplementation. Fish fed the ALGAE diet displayed increased mucus IgM levels, with a stronger response in females, indicating modulation of mucosal immune status.

This pattern was supported by proteomic analysis, processed against the turbot reference proteome, which retained 876 and 877 proteins in the first and second samplings, respectively. The ALGAE diet altered the skin mucus proteome in both sexes, with 165 and 51 differentially abundant proteins in females and males at the first sampling, and 56 and 47 at the second sampling. No significant diet × sex interaction was detected, indicating that the dietary effect was largely shared between sexes, although generally more pronounced in females.

Functional analysis of proteins, showing stable diet-associated responses across both sampling points, further supported the biological relevance of the ALGAE effect. In males, the dietary response was mainly associated with epithelial barrier integrity, cytoskeletal organization, protein folding and energy metabolism. In females, it was more strongly linked to proteostasis, autophagy, antigen processing and immune-related pathways.

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Together, the increased mucus IgM and the diet-associated proteomic shifts indicate that macro- and microalgae blend supplementation modulated mucosal condition in turbot breeders, supporting skin mucus as a useful non-lethal matrix for nutritional assessment under production/farming conditions.

Discussion

The results indicate that supplementation with a macro- and microalgae blend modulated mucosal immune condition in turbot breeders, with skin mucus IgM and proteomic profiles responding more clearly than plasma humoral parameters. Notably, one of the most affected humoral parameters by the ALGAE diet was the skin mucus IgM. This finding is of particular interest, as IgM in fish has been shown to possess neutralizing and agglutinating activities against viral and bacterial pathogens. Additionally, it activates the classical pathway of complement upon recognizing pathogens (Salinas, et al., 2021). Given the prevalence of IgM in coating a substantial portion of the microbiota on different mucosal surfaces, including the skin, it is plausible that IgM plays a relevant role in maintaining microbiota homeostasis at these sites

Although systemic humoral responses were limited, the mucus proteome revealed diet-associated changes in pathways related to epithelial integrity, protein homeostasis and immune function. These findings suggest that functional broodstock diets can influence mucosal condition under commercial farming conditions and highlight skin mucus proteomics as a useful tool for nutritional assessment in aquaculture.

Overall, this study supports the potential of algae-based dietary supplementation to modulate mucosal status in turbot breeders and reinforces the value of non-lethal approaches for monitoring broodstock condition during the reproductive season.

Acknowledgment

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RESPONSE OF THE MEDITERRANEAN MUSSEL (*Mytilus Galloprovincialis*) TO HEAT STRESS AND EFFECTS OF ENVIRONMENTAL FACTORS ON MICROPLASTICS PRESENCE IN MUSSEL FARM OF THE NORTHERN ADRIATIC SEA

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Introduction

Mediterranean mussel (*Mytilus galloprovincialis*) is a key economic species in Slovenian mussel farming; however, it is increasingly exposed to the impacts of climate change and microplastic pollution. Rising sea temperatures can induce physiological stress, reduced growth, and increased mortality, while microplastics represent an additional burden for filter-feeding organisms.

Methods

As part of the project, mussels were monitored at a mussel farm in Piran Bay between November 2023 and September 2024. Condition index, biomarkers of oxidative stress (superoxide dismutase, SOD; glutathione S-transferase, GST; and catalase), neurotoxicity (acetylcholinesterase, AChE), and mortality were assessed on a monthly basis. Simultaneously, seawater, sediment, and mussel samples were collected for microplastic determination using microscopic and Fourier-transform infrared spectroscopy (FTIR) analyses. To evaluate the influence of environmental factors on microplastic distribution, machine learning regression tree models (M5P algorithm) were applied to identify the key environmental drivers of microplastic occurrence in seawater and mussels.

Results

The results indicate pronounced physiological stress in mussels during the warmer part of the year. Increased activities of antioxidant defense enzymes and enhanced lipid peroxidation suggest elevated oxidative stress, probably associated with high seawater temperatures. The condition index decreased towards the end of summer, and high mortality rates were recorded in the sampled populations. A total of 203 microparticles were identified in water samples, of which 187 were microplastic particles. In sediments, altogether 80 microparticles were detected, while 660 microparticles were isolated from 400 analyzed mussels, including 319 microplastic particles. Textile fibers predominated across all sample types. The analysis revealed that wind direction, wave action, sea currents, and seasonality were the most important factors influencing the occurrence and distribution of microplastics in seawater and mussels. The contribution of plastic aquaculture infrastructure to microplastic contamination appeared to be limited, as only a small number of particles could potentially be linked to farming materials. Most detected particles originated from other, unidentified sources, including potentially wastewater-derived inputs and other land-based activities.

Conclusions

The findings confirm that the combined effects of thermal stress and microplastic exposure can represent a significant challenge for sustainable mussel farming in the northern Adriatic Sea. Thermal stress is currently a major pressure affecting mussel physiological condition and survival. Although microplastics were consistently detected in seawater, sediments and mussels, their specific biological effects relative to other environmental stressors remain insufficiently understood and require further investigation. Monitoring physiological biomarkers together with microplastic contamination provides a valuable tool for early risk detection and for developing adaptation strategies for aquaculture under changing environmental conditions.

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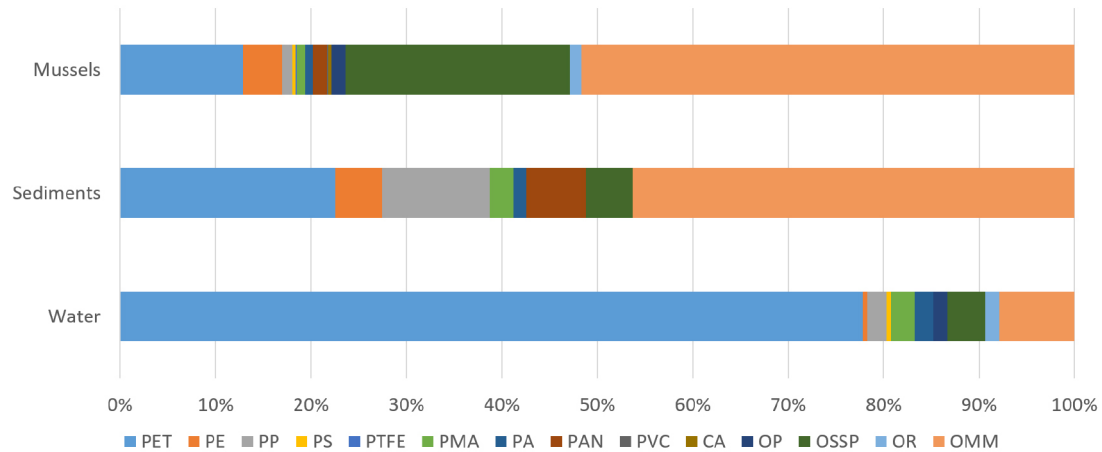


Figure 1: Distribution of microparticles identified in water, sediment, and mussel samples according to their chemical composition (PET – Polyethylene terephthalate; PE – Polyethylene; PP – Polypropylene; PS – Polystyrene; PTFE – Polytetrafluoroethylene; PMA – Poly(methyl acrylate); PA – Polyamide; PAN – Polyacrylonitrile; PVC – Polyvinyl chloride; CA – Cellulose acetate; OP – Other plastics; OSSP – Other semi-synthetic polymers; OR – Other rubber; OMM – Other man-made materials)

Acknowledgment

This work was carried out within a project Impact of environmental stress on mussel health and recruitment, and improvements in farming practices due to microplastics and climate changes (V1-2205), founded by Ministry of Agriculture and Slovenian Research and Innovation Agency.

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IN VITRO IMMUNOMODULATORY POTENTIAL OF *Palmaria palmata* FOR FUNCTIONAL FEED DEVELOPMENT

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Introduction

Palmaria palmata is a protein-rich red seaweed containing essential amino acids and bioactive compounds with potential applications in aquaculture nutrition and fish health. In this study, liquid extracts enriched in soluble compounds were obtained through mechanical fractionation of *P. palmata*. The recovered fractions were characterized for their lipid and amino acid composition and evaluated for cytotoxic and immunomodulatory response in salmon head kidney-derived SHK-1 cell line (*Salmo salar*) cell lines as a first step towards assessing their suitability as functional ingredients for aquaculture feed applications.

Methodology

P. palmata was collected from Mørkvedbukta, Bodø (Norway) in October 2023 and maintained in a flow-through seawater system until processing. Biomass was mechanically fractionated using an Angel twin-screw press at 8°C under two treatments (i) **S1S3**: Surface water removed before pressing, (ii) **S2S3**: Quick freshwater wash followed by pressing. The liquid fractions were filtered through a 500 µm sieve and freeze-dried. Biochemical characterization included dry matter, protein, lipid, ash, pH, salinity, and electrical conductivity measurements. Amino acid composition of the liquid fractions was analyzed. For biological evaluation, cell viability was determined using the MTT assay, and cytotoxicity was expressed as the concentration required to reduce cell viability by 50% (CC₅₀). The potential immunomodulatory activity of the extracts was evaluated using the SHK-1 cell line (*Salmo salar*), with extract concentrations ranging from 100 to 500 µg mL⁻¹ and an exposure period of 24 h.

Results

S2S3 showed higher protein content (156.11 ± 17.6 mg/g) than S1S3 (109.79 ± 1.9 mg/g), despite lower salinity, EC, DM, and ash contents. Both extracts contained a broad range of amino acids, with glutamic acid, aspartic acid, proline, glycine, serine and alanine being the major components. **S2S3** consistently showed higher levels of almost all amino acids than **S1S3**, with the total measured amino acid content increasing from approximately 109.8 to 156.1 (about 42% higher) in S2S3.

Both S1S3 and S2S3 extracts increased MTT-based cell viability/metabolic activity compared with the control, indicating that the extracts were not strongly cytotoxic at the tested concentrations. S1S3 showed the strongest immunomodulatory response, particularly at 500 µg/mL (S1S3-2), with a marked increase in IL-1β (~47-fold) and IL-10 (~5.7-fold) relative to the control. Whereas 100 µg/mL S1S3 treatment (S1S3-1) produced little or only modest cytokine induction, suggesting a concentration-dependent response. In contrast, **S2S3 treatments** showed little change in IL-1β or IL-10 expression compared with the control and did not reproduce the strong response observed for S1S3. The LPS positive control strongly induced IL-1β, confirming the responsiveness of the assay; however, the S2S3 treatments did not show a comparable response.

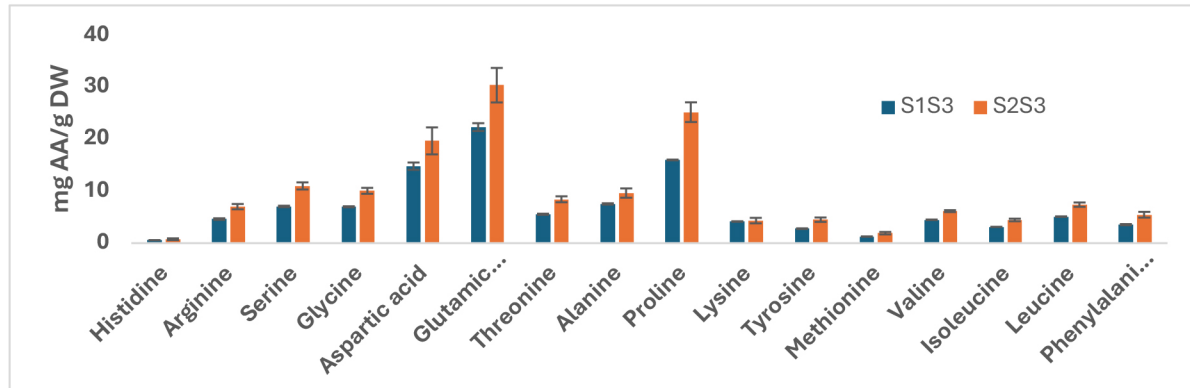
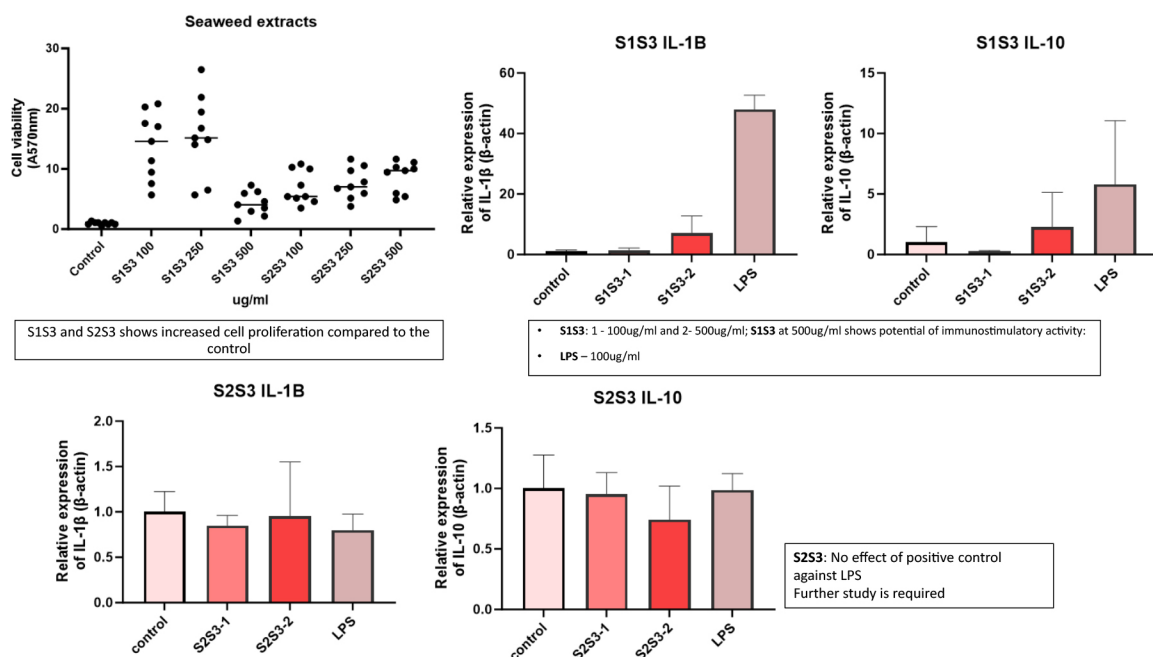
Overall, S1S3 at 500 µg/mL demonstrated the clearest immunostimulatory potential, whereas the S2S3 extract showed limited immunomodulatory activity under the tested conditions. The liquid fractions contained diverse amino acids, dominated by glutamic acid, aspartic acid, proline, glycine and serine. S2S3 exhibited a higher overall amino acid content than S1S3; however, this did not translate into greater immunostimulatory activity in SHK-1 cells. In contrast, S1S3 at 500 µg mL⁻¹ markedly increased IL-1β and IL-10 expression, suggesting that the observed cellular response was not directly governed by total amino acid abundance. The activity may instead reflect differences in specific bioactive peptides/proteins or other co-extracted compounds generated or retained under the S1S3 processing conditions.

Preliminary *in vitro* screening identified *P. palmata* S1S3 as a promising candidate for further functional-feed development, based on its immunomodulatory response in SHK-1 cells. Further compound characterization, dose optimisation and feeding trials are required to validate its functional potential.

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Table 1 Physicochemical characteristics and proximate composition of liquid fractions from *P. palmata*

Extracts	pH	Salinity (PSU)	EC (g/L)	DM %	Total protein (mg/g)	Lipid (%)	Ash (%)
S1S3	6.246	33.2	51.2	11.02±0.11	109.79±1.9	1.4	31.35±0.33
S2S3	6.638	17.2	29	9.19±0.15	156.11±17.6	1.6	27.86±1.55

**Figure 1.** Amino acid profile of liquid fractions from *P. palmata* (mean± standard error; n=3)**Figure 2.** Cellular viability and immunostimulatory activity of *P. palmata* liquid extracts in SHK-1 fish cell lines. Values are presented as mean ± standard error (n = 3).

RECOVERING MORE WITH LESS: A SUSTAINABLE APPROACH TO PHYCOERYTHRIN RECOVERY FROM *Palmaria palmata*

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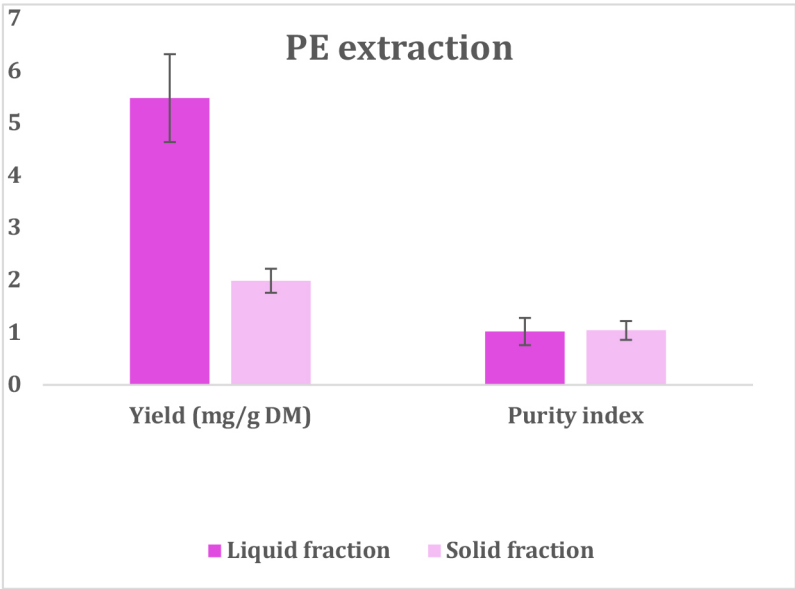
Introduction

There is growing interest in simple and resource-efficient recovery strategies that maximize pigment yield while minimizing chemical inputs and processing intensity. Mechanical fractionation using twin-screw press offers a promising approach by releasing soluble intracellular compounds into a liquid fraction while simultaneously generating a solid fraction that can be further valorized. In this study, freshly harvested *P. palmata* biomass was mechanically fractionated prior to ammonium sulfate precipitation, dialysis, and freeze-drying to produce a phycoerythrin. This approach aims to maximize R-PE recovery through low-input processing while reducing chemical consumption and energy requirements compared with conventional extraction methods. The distribution and recovery of R-PE within the generated process streams were evaluated to assess the potential of mechanical fractionation as a primary recovery step in phycoerythrin extraction.

Methodology

Fresh *Palmaria palmata* (2 kg) was harvested from Mørkvedbukta, Bodø, Norway, during low tide on 27 December 2023. The biomass was cleaned of debris, briefly rinsed with freshwater to remove surface salts, and mechanically fractionated using a twin-screw press to obtain liquid and solid fractions, with the mass balance of each fraction recorded. The liquid fraction was subjected to ammonium sulfate precipitation (25%, w/v; 1:4 w/v) for 6 h at 4°C, whereas the solid fraction was treated with 25% (w/v) ammonium sulfate and incubated for 12 h at 4°C. After centrifugation (4000 rpm, 20 min), supernatants and pellets were collected. The solid fraction underwent a second precipitation step by increasing ammonium sulfate saturation from 30 to 60%, followed by 4 h incubation at 4°C and centrifugation under the same conditions. The resulting pellets were dissolved in 0.05 M phosphate buffer (pH 6.8), purified by gel filtration, and dialyzed against distilled water using a 12–14 kDa molecular weight cut-off membrane to remove residual salts and low-molecular-weight impurities (Ganesan et al., 2020; Ramu Ganesan et al., 2022). Elution was performed using stepwise NaCl concentrations (0.05–0.35 M) to determine the optimal ionic strength for achieving the highest R-PE purity. The dialyzed extract was subsequently freeze-dried to obtain a phycoerythrin-enriched powder. The purity index (PI) was calculated as the ratio of absorbance at 564 nm (A564), corresponding to the maximum absorbance of R-PE, to absorbance at 280 nm (A280), representing total protein content (Bastos-Filho et al., 2025).

Figure 1: PE recovery and purity of fractionated *Palmaria palmata*



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Results

Mechanical fractionation of *P. palmata* generated a PE-rich liquid stream and a solid fraction. The dry matter content of liquid fraction was 9.02% and 16.8% DM in solid fraction. The liquid fraction yielded 0.444 g of PE-enriched powder with a purity index (PI) of 1.02, whereas the extract recovered from the residual solid biomass showed a similar PI of 1.04. The extraction efficiency (%) shows 52.9% recovered PE was obtained in the liquid fraction, while 47.1% was obtained from solid fraction, indicating preferential partitioning of water-soluble phycoerythrin into the expressed liquid stream. This represents PE yield from the liquid fraction was 5.48 mg/g DM in the liquid stream was substantially higher than that obtained from the residual solid biomass of 1.99 mg/g DM. These findings demonstrate that the screw-press fractionation step served as an effective primary recovery process, concentrating PE into a relatively small liquid-derived biomass fraction.

Conclusion

Phycoerythrin-enriched extracts obtained from both liquid and solid fractions exhibited purity indices of 1.02 and 1.04, respectively, corresponding to food-grade phycoerythrin. These values are comparable to the PI reported for *Grateloupia turuturu* (1.07) but lower than highly purified R-PE preparations from *Pyropia yezoensis* (2.8-5.4), indicating successful enrichment rather than complete purification of the pigment. Mechanical fractionation and simple downstream processing, avoiding energy-intensive technologies and multiple purification steps. In addition, recovering half of the PE-enriched material directly from the expressed liquid stream reduces solvent use, processing steps, and operational costs, making it attractive for scalable seaweed biorefinery applications.

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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FOOD-COMPATIBLE FRACTIONATION OF *Ascophyllum nodosum* FOR FUCOIDAN-RICH EXTRACT RECOVERY AND CASCADING BIOMASS VALORISATION

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Introduction

Brown seaweeds are rich in sulphated polysaccharides, minerals, proteins, and other bioactive compounds suitable for cascading biorefinery. *Ascophyllum nodosum*, an abundant North Atlantic species, contains fucoidan, laminarin, and mannitol with potential food and feed applications. Efficient utilisation requires fractionation strategies that recover target compounds while enabling further valorisation of the remaining biomass. Here, a food-compatible process using water and citric acid was applied to recover fucoidan- and alginate-rich fractions and retain residual biomass for further use. Material distribution, concentration efficiency, and recovery were evaluated across the process, which recovered 57.3% of the initial dry biomass as value-added products.

Methodology

A. nodosum (1.082 kg DW) was soaked in cold fresh water for one hour to remove surface salt. The biomass was then subjected to citric acid extraction using 0.1 M citric acid at a biomass-to-liquid ratio of 1:15 (w/v), with a total initial process input of 16.37 kg. The extraction mixture was heated to 80 °C for 2 h and subsequently cooled before separation by muslin-cloth filtration, yielding a fucoidan-rich liquid extract and wet residual biomass. The recovered filtrate (14.0 L) was concentrated to 4.0 L at 40 °C, corresponding to a 71.4% reduction in volume and a 3.5-fold concentration factor. The concentrated extract was subsequently treated with 1% CaCl₂ at 4 °C overnight for clarification. Following treatment, the mixture was centrifuged at 4500 rpm for 20 min, and the resulting supernatant was subjected to different downstream purification approaches, including dialysis using a cellulose membrane with a nominal MWCO of ~14 kDa, DEAE-Sepharose Fast Flow chromatography, activated charcoal treatment, and isopropanol precipitation at a 1:2 extract-to-isopropanol volume ratio. The residual wet biomass was further processed using two alkaline extraction conditions with 1% and 2% Na₂CO₃ (w/v) to recover alginate-rich compounds and support further valorisation of the residual biomass.

Results: Among the purification strategies, dialysis (7A) yielded the highest fucoidan enrichment, containing 410 mg/g fucoidan (41.0%), compared with 212 mg/g (21.2%) in the isopropanol (IPA)-precipitated fraction (7D) and approximately 11% in the Fast Flow chromatography filtration (7B) and charcoal-treated (7C) fractions. Thus, IPA precipitation maximized extract recovery, whereas dialysis achieved the highest fucoidan purity. The fractions also differed in their uronic acid content, which ranged from 372 to 798 mg/g DW in the order 7A > 7D > 7B > 7C. Mineral analysis further confirmed the purity of the recovered fractions, with toxic elements remaining below detection limits or present only at trace levels. ICP-OES analysis revealed purification-dependent differences in mineral composition. The dialysis fraction exhibited the highest sulphur (49.6 g/kg) and calcium (up to 65.1 g/kg) concentrations, whereas 7B and 7C fractions retained higher levels of sodium and iron. Trace amounts of Cd (0.01–0.29 mg/kg) and Pb (0.29–0.31 mg/kg) were detected in selected samples. The residue biomass has 4% and 14% of alginate recovered after alkali treated. Overall, the purification strategy strongly influenced both fucoidan enrichment and mineral composition, highlighting its importance in producing fucoidan fractions with different compositional characteristics. Cascading biorefinery processing converted 57.3% of the initial dry biomass (619.6 g from 1082 g DW) into value-added fucoidan- and alginate-rich products, while 20.2% remained as residual biomass and 22% accounted as processing loss.

Acknowledgement: This study as part of the project “AquaUP - Aquafeed Upcogreded: 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.

ENHANCING WATER STABILITY IN AQUAFEED PRODUCTION: TRIAL RESULTS

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Introduction

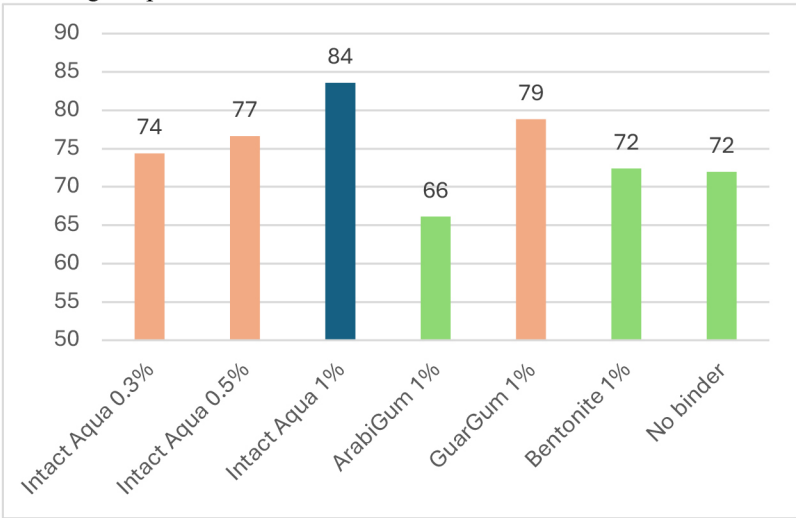
Producing aquafeeds with adequate water stability and pellet durability remains a key challenge in aquaculture. Feed formulations typically combine macro ingredients such as marine proteins, plant-based raw materials, and starch sources, supported by micro ingredients including minerals, phosphates, organic acids, and binders. Water stability is particularly important for species that feed slowly or at the bottom, such as shrimp, carp, catfish, and many marine benthic fish. These species require pellets to remain intact in water for extended periods to allow detection, handling, and consumption. Insufficient pellet stability leads to high nutrient leaching, reduced feed efficiency, and deterioration of water quality (Obaldo et al., 2002). Therefore, binders play a crucial role in maintaining pellet integrity, improving durability, and supporting consistent feed manufacturing processes.

In practice, a wide range of binders is used in aquafeed production, each exhibiting different functional properties during processing. Despite their widespread use, direct comparisons under controlled conditions remain limited, and optimizing binder selection remains an important focus in feed technology (Behnke, 2001).

Materials and Methods

A laboratory study conducted at the Borregaard Research Center (Norway) evaluated the effect of a newly developed multifunctional pelleting aid for aquafeed on water stability. The basal formulation consisted of wheat (30%), fishmeal (25%), and soybean meal (45%). Diets included a control without binder, 1% bentonite, 1% guar gum, 1% arabic gum, and the new multifunctional binder at 0.3%, 0.5%, and 1% inclusion levels. Pelleting was performed using a Münch Edelstahl Lab Pellet Press RMP 250/250. Conditioning was achieved by direct steam injection at 2 bar, targeting a temperature range of 90–100°C. After pelleting, samples (2.5 kg per treatment) were collected and cooled at ambient temperature under forced air ventilation for 20 minutes. This step ensured proper pellet hardening and stabilization prior to testing. Water stability was assessed by soaking pellets in water for 2 hours, removing fines, and determining the dry weight of the remaining intact pellets.

Figure 1. Water stability of pellets after 2 hours of soaking. Green bars indicate very weak pellets, orange bars indicate soft pellets, and blue bars indicate good pellet structure.



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Results

Water stability varied significantly depending on binder type and inclusion level. The new multifunctional binder showed a clear dose-dependent improvement, increasing from 74 (0.3%) to 77 (0.5%) and reaching the highest value of 84 at 1% inclusion. Guar gum (79) was the second-best performer, followed by bentonite (72), which showed no improvement compared to the control (72). Arabic gum resulted in the lowest stability (66).

Discussion

The superior performance of the new aquafeed binder at 1% inclusion indicates enhanced binding capacity and improved cohesion within the pellet matrix. The clear dose-dependent response suggests that increasing inclusion levels strengthens inter-particle interactions, resulting in greater resistance to disintegration during prolonged water exposure. This is particularly relevant for bottom-feeding and slow-feeding species, where pellet integrity must be maintained over extended periods.

In addition to its binding functionality, the product can be considered a multifunctional binder, as previous processing trials have demonstrated its ability to improve pelleting efficiency. Reductions in electrical load during pelleting and increases in production throughput have been observed, indicating improved material flow and reduced mechanical resistance during processing (data not shown). These effects are likely related to improved lubrication and matrix plasticization during conditioning and pelleting.

The differences observed between binders further highlight the importance of binder type. Guar gum, a galactomannan polysaccharide, is known for its high viscosity and water-binding capacity, contributing to improved pellet cohesion, although its effectiveness depends on hydration and processing conditions (BeMiller, 2019). Bentonite, a clay-based binder, primarily improves physical structure through particle packing and absorption but may have limited impact on water stability compared to organic binders (Behnke, 2001). In contrast, arabic gum, a highly soluble polysaccharide, tends to disperse rapidly in water, which can reduce pellet integrity under prolonged immersion, as reflected in the lower stability values observed in this study.

Processing conditions, including moisture content, temperature, and starch gelatinization, play a critical role in pellet formation and durability (Cheng et al., 2023; Novriadi et al., 2025). These factors influence matrix formation and inter-particle bonding, directly affecting pellet resistance to water-induced disintegration. The consistent performance of the new multifunctional aquafeed binder across treatments indicates that it effectively complements these processing conditions, enhancing pellet consolidation and structural integrity.

Overall, the results confirm that both binder type and inclusion level are key determinants of pellet water stability. The improved performance of the new multifunctional binder has direct implications for feed efficiency, nutrient retention, and environmental sustainability in aquaculture systems (Lall & Dumas, 2015).

Conclusion

The new multifunctional aquafeed binder significantly improved pellet water stability, with optimal performance observed at 1% inclusion. Its effectiveness highlights its potential as a sustainable solution for aquafeeds, particularly for bottom-feeding and slow-feeding species where pellet integrity is critical.

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GROWTH PERFORMANCE OF NILE TILAPIA SELECTED FOR RESISTANCE TO *Francisella orientalis*

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Introduction

Francisellosis, caused by *Francisella orientalis*, is an important bacterial disease affecting Nile tilapia farming worldwide. Selective breeding has emerged as a sustainable strategy to improve disease resistance; however, concerns remain regarding potential trade-offs between disease resistance and productive performance. This study evaluated the reproductive and growth performance of four Nile tilapia (*Oreochromis niloticus*) G1 families genetically selected for resistance to *F. orientalis* and compared them with three commercial strains widely used in Brazil.

Material and Methods

The genetic material evaluated consisted of seven groups of Nile tilapia (*Oreochromis niloticus*): four families genetically selected for resistance to *Francisella orientalis* (designated CCD 1, CCD 2, CCD 3, and CCD 4), originating from the G1 generation of the breeding program described by Garcia et al. (2025a/b), and three commercial strains widely used in Brazilian aquaculture, adopted as commercial control strains (A, B, and C). The trial was conducted for 60 days in hapas installed in earthen ponds, including nursery and juvenile phases. Reproductive performance was assessed through egg production per female, while growth performance was evaluated through body weight, total length, weight gain, specific growth rate and Fulton's condition factor. Statistical analyses were performed using Welch's analysis of variance, Games-Howell multiple comparisons and non-parametric tests ($P < 0.05$).

Results

Commercial strains showed greater reproductive uniformity, whereas selected families exhibited variability in egg production, ranging from 5 to 30 mL female⁻¹. Significant differences in growth performance were observed among groups. The selected family CCD3 presented superior performance during both nursery and juvenile phases. The CCD3 family achieved the highest body weight (52.30 ± 7.71 g), outperforming the lowest-performing commercial strain by more than 41%. Fulton's condition factor indicated adequate nutritional status and welfare for most groups. These findings demonstrate that selection for resistance to *F. orientalis* did not compromise productive performance and may simultaneously improve disease resistance and growth traits. The selected family CCD3 combined superior growth performance with resistance-related traits, demonstrating the potential of selective breeding programs to improve both productivity and health in Nile tilapia aquaculture.

Conclusion

The G1 generation Nile tilapia families selected for resistance to *Francisella orientalis* demonstrated functional reproductive viability and zootechnical performance comparable to, or superior to, that of established commercial strains during the fingerling and juvenile phases. The superior weight gain observed in family CCD 3 demonstrates that selection for immunological traits does not necessarily result in a severe trade-off affecting early growth performance in this species. Finally, the reproductive heterogeneity observed among the selected families highlights the need to adopt multi-trait selection indices in future generations in order to balance gains in disease resistance with the spawning uniformity required by the aquaculture industry.

Acknowledgment

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Table 1. Final body weight of selected and commercial Nile tilapia families during the juvenile phase.

Family/Strain	Final Weight (g)
CCD1	30.39 ± 6.04
CCD2	26.66 ± 5.89
CCD3	52.30 ± 7.71
CCD4	23.41 ± 5.80
Commercial A	30.27 ± 7.10
Commercial B	33.97 ± 4.87
Commercial C	21.84 ± 5.26

SCALE-Net: SHAPE AND CONTOUR-AWARE LIGHTWEIGHT ESTIMATION FOR UNDERWATER FISH DETECTION AND MONITORING IN AQUACULTURE ENVIRONMENTS

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The rapid adoption of underwater imaging systems in aquaculture has created increasing demand for automated fish monitoring technologies. Current vision-based detection methods can effectively identify and locate fish targets, but they often provide limited information about fish morphology, restricting their usefulness for applications such as stock assessment, growth evaluation, and behavioral monitoring.

To address this challenge, we propose SCALE-Net, a lightweight shape-aware Lightweight framework that combines fish detection with contour reconstruction for enhanced underwater observation. The proposed method is designed to extract representative structural features from underwater imagery and estimate anatomical landmarks that describe fish body geometry. These structural cues are subsequently used to reconstruct fish contours, enabling the generation of more informative target representations than conventional bounding-box outputs.

SCALE-Net incorporates an adaptive feature learning strategy to improve robustness under challenging underwater conditions, including low visibility, illumination variations, background interference, and partial occlusions. The lightweight architecture maintains computational efficiency while supporting accurate detection and shape estimation, making it suitable for deployment in practical aquaculture monitoring systems.

Experiments conducted on public underwater fish datasets demonstrate that the proposed framework achieves reliable fish detection performance while providing detailed contour information across diverse observation scenarios. By integrating target localization with morphological representation, SCALE-Net offers a practical tool for intelligent aquaculture management, fish population assessment, and long-term automated monitoring of aquatic environments.

Graphical Abstract

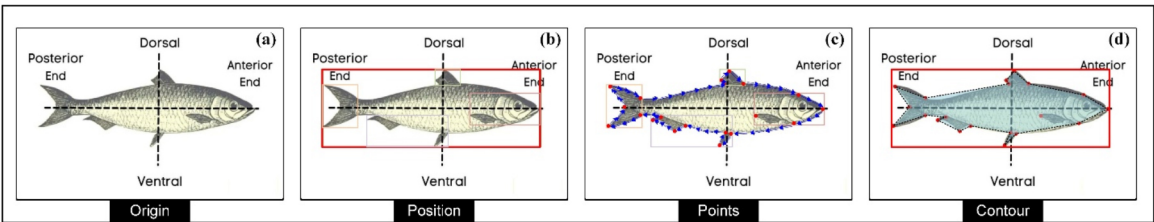


Figure 1 Pipeline of the SEALNet

DigiATLA: BUILDING DIGITAL COMPETENCIES FOR PRECISION AQUACULTURE

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Background and Rationale

Atlantic aquaculture faces a set of shared challenges related to environmental sustainability, production efficiency, the need for new technical skills, and the uneven adoption of digital tools. The digitalisation gap particularly affects small and medium-sized enterprises (SMEs), which often continue to rely on low levels of automation and limited data integration. These challenges are further intensified by competitive pressures, the impacts of climate change, and the need to align production practices with European policies supporting a more sustainable blue economy. In this context, strengthening digital competencies and facilitating access to practical digital solutions is essential to support the modernisation and long-term resilience of the sector.

Project Concept and Objectives

DigiATLA was developed in response to these needs, with the objective of reinforcing digital capacities and promoting the adoption of Industry 4.0 solutions in Atlantic aquaculture. The project brings together seven organisations from Portugal, Spain, France, and Ireland, combining academic expertise, technical know-how, and strong links to the aquaculture production sector. The DigiATLA approach integrates digital tools, nutritional modelling, data analysis techniques, and environmental assessment methodologies to deliver applicable and adaptable solutions across different production contexts. The project is structured around three core pillars: training and capacity building, practical demonstration of digital tools, and awareness-raising activities targeted at aquaculture stakeholders. Together, these pillars aim to support informed decision-making and accelerate the digital transition of the sector.

Implementation Strategy and Activities

The project implementation is organised into three complementary blocks of activities. First, DigiATLA develops training content focused on aquaculture data digitalisation, digital nutritional tools, and environmental assessment through Life Cycle Assessment (LCA). Three dedicated training modules are produced, including presentations, practical exercises, case studies, and tutorials, and are made available online via a Moodle platform.

Second, practical sessions, workshops, and courses are delivered both online and on-site in Portugal, Spain, France, and Ireland, targeting students, professionals, and technical staff. These activities include the applied use of FEEDNETICS (Soares et al., 2023), a digital nutritional model that allows simulation of growth performance, feed efficiency, and nutrient utilisation under different production scenarios. In parallel, MEANS-InOut (Auberger et al., 2018) is used to estimate material and energy flows and calculate environmental indicators supporting LCA-based impact assessments. Intensive training courses and interactive workshops further promote knowledge exchange between academia and industry. Third, pilot actions are conducted through experimental trials with gilthead seabream (*Sparus aurata*) and Atlantic salmon (*Salmo salar*) in facilities located in Portugal and Ireland. These trials generate data on growth, feed efficiency, welfare, nutrient discharges, and environmental performance. The collected data are integrated into digital models to generate comparative scenarios and practical examples that are subsequently incorporated into training activities.

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Expected Outcomes and Sectoral Impact

DigiATLA is expected to significantly enhance digital skills among students and aquaculture professionals. The multilingual online repository will ensure accessibility and continuity of training beyond the project lifetime. Pilot actions are expected to demonstrate improved feeding management, a more accurate understanding of growth and welfare drivers, and potential reductions in nutrient discharges through the application of predictive digital tools. The use of LCA is expected to support the identification of production strategies with lower environmental impact. From a sectoral perspective, the project aims to facilitate the adoption of digital tools, supporting the transition towards more efficient, sustainable, and resilient aquaculture practices. Collaboration among international partners contributes to the harmonisation of methodologies and fosters durable innovation networks. In the medium term, DigiATLA is expected to enhance competitiveness, strengthen climate resilience, and improve the integration of environmental criteria into decision-making processes in Atlantic aquaculture.

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INTEGRATED TRANSCRIPTOMIC AND PHYSIOLOGICAL RESPONSES TO STRESS IN SENEGALESE SOLE LARVAE FED AN ALGAE-BASED FUNCTIONAL DIET

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Introduction

Early life stages are critical periods marked by high vulnerability to stressors and constituting a critical bottleneck in marine aquaculture. In Senegalese sole (*Solea senegalensis*), unavoidable handling events and bacterial challenges often elicit acute stress responses that disrupt immune regulation and metabolic homeostasis, compromising health and survival. Enhancing larval resilience under these conditions is therefore essential to improve health management and production efficiency. Functional diets have gained attention as a strategy to modulate larval stress responses by supplying bioactive compounds that act on immune, antioxidant and stress-related pathways rather than directly promoting growth. Among functional ingredients, micro- and macroalgae represent particularly attractive candidates due to their complex biochemical composition. Collectively, these components may support mucosal integrity, reduce oxidative damage and promote coordinated systemic responses during acute stress exposure. In this context, the present study investigated whether a short functional feeding period with an algae-based blend modulates stress resilience in sole larvae exposed to combined transport and bacterial challenge. By integrating survival outcomes with humoral, oxidative and gut–liver transcriptomic responses, this work aims to characterize the impact of algae-enriched microdiets on the biological response to stress during early development of sole.

Methodology

The experimental trial was conducted in SPAROS experimental facilities (Olhão, Portugal). Sole larvae were reared in a RAS system, in triplicate tanks under controlled zootechnical conditions, and fed the experimental diets from 67 to 72 DAH: a commercial-like control diet (CTRL) formulated with high quality ingredients for a high performance, a negative control (CTRL-) formulated with high quality ingredients for a medium performance and a functional diet containing *Ulva* and *Tetraselmis* biomasses (ULVTET). After the feeding period, larvae were transported to a second facility (CIIMAR, Porto, Portugal) and exposed by bath to *Tenacibaculum maritimum* (1×10^4 CFU/mL), followed by a 14-day mortality follow-up. Two sampling points were considered: Pre-stress and Post-stress (18 h after pathogen exposure). Survival was analysed using Kaplan–Meier curves and a Cox hazard model. Humoral indicators and antioxidant enzymes activity were measured to assess the immune response and oxidative stress. Gut and liver RNA-seq was conducted (differentially expression analysis with FDR < 0.01) to assess larvae transcriptome response.

Results and Discussion

Transport followed by bacterial challenge induced a pronounced stress response in Senegalese sole larvae across all dietary groups, reflected by coordinated molecular, physiological and survival outcomes. Independent of diet, the gut transcriptomic response after challenge was characterized by a marked downregulation of cell cycle progression, DNA replication and repair, and proteasome-related pathways, indicating a conserved shift away from proliferative functions. Concomitantly, immune- and stress-responsive signaling pathways were activated, although their biological organization differed substantially between diets. In larvae fed the CTRL diet, the post-stress gut response was dominated by strong upregulation of cytokine and interleukin signaling, chemokine-mediated pathways and MAPK/AKT signaling, suggesting a robust inflammatory activation. This was associated with extensive liver remodeling, with enrichment of ER–Golgi trafficking, ribosome biogenesis and RNA processing, consistent with a broad systemic metabolic and secretory reprogramming. CTRL- fed larvae exhibited a similarly extensive transcriptional response but with gut enrichment additionally involving developmental and structural pathways, including ciliary assembly and morphogenetic processes. This molecular profile, together with less favorable oxidative stress balance and significantly increased mortality risk during the infection follow-up, indicates a reduced capacity to cope with the combined stressors. In contrast, larvae fed the ULVTET algae blend displayed a distinct resilience-associated signature. Although gut immune activation was maintained, with enrichment of cytokine–cytokine receptor interactions, chemokine signaling, eicosanoid/prostanoid receptors and ion

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homeostasis, hepatic transcriptomic disruption was markedly attenuated. Liver responses were more targeted, involving upregulation of protein processing, membrane trafficking and innate immune recognition pathways, together with unfolded protein response mechanisms. This coordinated gut–liver response aligned with improved oxidative stress regulation. While survival probability after challenge of ULVTETfed larvae was not statistically higher than that of the CTRL diet ($p = 0.9400$), Cox regression analysis revealed similarly lower mortality risk, in contrast with the CTRL- group, which was significantly higher than CTRL ($p = 0.0019$). Overall, the integrated data indicate that short-term functional feeding supports stress adaption and promotes a more regulated immune-metabolic response, limiting systemic destabilization and enhancing stress resilience in sole larvae.

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SKIN-DERIVED MULTIOMICS BIOMARKERS ENABLE MINIMALLY-INVASIVE STRESS MONITORING IN EUROPEAN SEABASS AQUACULTURE

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Introduction

The increasing exposure of farmed fish to acute and chronic stressors, intensified by climate change and production intensification, highlights the urgent need for reliable biomarkers that enable early, noninvasive assessment of stress maladaptation and health. In European seabass (*Dicentrarchus labrax*), common aquaculture practices such as transport and prolonged temperature challenges can disrupt immune function and homeostasis. The fish skin interface represents a strategic target for biomarker discovery, acting both as the first biological barrier against environmental stressors and as an accessible matrix for minimally invasive sampling. In this context, we are identifying biomarker panels based on the skin interface to capture integrated stress responses under both acute and chronic challenge conditions. Multiomics data were jointly analysed through integrative modeling approaches, enabling the identification of composite biomarker signatures that reflect coordinated regulation across molecular levels relevant to stress resilience. These signatures are being validated using an independent trial, assessing the robustness and transferability of the identified biomarkers in a separate biological context. Together, this work advances the development of biologically grounded, minimally invasive biomarker tools with direct applicability for stress monitoring and welfare-oriented decisionmaking in seabass aquaculture.

Methodology

European seabass juveniles (~65g) were subjected to two independent stress models, in INMAR (Cádiz, Spain), representative of common aquaculture scenarios: an acute transport stress and a chronic thermal challenge. For the acute trial, fish were exposed to transport conditions (4h) designed to mimic standard commercial practices, with skin tissue and skin mucus samples collected from the control group and the challenged group (n = 3 tanks/group). For the chronic trial, fish were maintained under control (19 °C) or elevated temperature conditions (27 °C) for four weeks (n = 3 tanks/group), after which skin and skin mucus samples were collected. Skin transcriptomic profiles were generated by RNA sequencing, while miRNA profiles were obtained from skin mucus. Differential expression analyses were conducted (edgeR; FDR < 0.05) to identify stress-responsive molecular features associated with each challenge. To capture coordinated stress responses across molecular layers, transcriptomic and miRNAs datasets were integrated using Multi-Omics Factor Analysis (MOFA+), enabling the extraction of latent factors and composite biomarker signatures shared across acute and chronic stress conditions. An independent validation is currently being performed using skin samples from a separate European seabass trial conducted at RIASEARCH Lda (Murtosa, Portugal). In this trial, fish (~60g) were exposed to thermal stress (26-28 °C) for five weeks (n = 4 tanks). Targeted qPCR analyses of selected genes and miRNA biomarkers are being carried out in skin samples to assess the robustness, reproducibility, and transferability of the identified biomarker panel in an independent biological context.

Results and Discussion

Both acute transport and chronic thermal challenges elicited marked molecular responses in the skin interface of European seabass, reflected in distinct yet partially overlapping transcriptomic and miRNAomic signatures. Acute stress primarily induced rapid modulation of genes, i.e., 1064 differentially expressed genes (DEGs), and 29 miRNAs, whereas chronic thermal exposure induced the dysregulation of 1427 genes and 10 miRNAs. Despite the different temporal dynamics of the two stress models, shared genes were consistently enriched in pathways associated with immune signaling such as cytokine signaling, stress regulation pathways such as MAPK and FoxO, along with processes linked to cell migration, apoptosis, wound healing and barrier maintenance, reinforcing the biological relevance of the skin interface as both a frontline defense tissue and a sensitive readout of stress. Moreover, several downregulated processes related to DNA damage response, DNA repair, and cell cycle were observed, suggesting the induction of senescence-related molecular programs, which if prolonged can be maladaptive and have potential implications for longterm tissue function and resilience.

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Multimomics MOFA+ modeling disentangled shared and challenge-specific latent factors, demonstrating that integrated molecular patterns provided stronger discrimination between stressed and non-stressed fish than single-omic markers alone and enabling the identification of composite biomarker signatures (top weighted features) that were conserved across stress models. The analysis of an independent cohort will allow us to assess the model's performance and biomarker reproducibility. The validation by qPCR will further demonstrate the feasibility of translating multi-omics discoveries into simplified, assay-ready formats suitable for more routine monitoring. Overall, these findings reinforce the skin interface as a biologically meaningful and minimally invasive source of biomarkers and support the development of integrated biomarker panels for early stress detection and welfare-oriented decision-making in European seabass aquaculture.

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***Salicornia ramosissima* BIOACTIVES AS A FUNCTIONAL INGREDIENT FOR DISEASE RESISTANCE IN EUROPEAN SEABASS (*Dicentrarchus labrax*)**

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Introduction

Salicornia ramosissima is an edible, highly salttolerant halophyte species that has gained attention due to its diverse bioactive compounds, particularly polyphenols (phenolic acids, hydroxycinnamic acids and flavonoids), which are associated with antioxidant, antiinflammatory, and antimicrobial activities (Silva *et al.*, 2021; Nájár *et al.*, 2023; Hulkko *et al.*, 2023). Evidence for its use in fish and shrimp health management is emerging but remains limited. Dietary inclusion of *S. ramosissima* biomass in *Penaeus vannamei* juveniles revealed favourable but transient effects on immune and oxidative status (RamosPinto *et al.*, 2024). The authors also highlighted its potential contribution to circular bioeconomy and environmental sustainability, as the plant can grow in harsh environments without competing with fresh water species (RamosPinto *et al.*, 2024). However, Machado *et al.* (2025) reported that inclusion of 10% nonfood *S. ramosissima* fraction did not alter growth or immune status but, improved leucocyte response and upregulation of key immunerelated genes in seabass exposed to an inactivated pathogen. Despite these promising findings, important gaps remain. The effects of purified and unpurified bioactive fractions of *S. ramosissima* on seabass (*Dicentrarchus labrax*) performance and resilience are poorly understood. Therefore, the present study explored the potential of *S. ramosissima* bioactive compounds for health management and disease resistance in European seabass following challenge with *Photobacterium damsela* subsp. *piscicida*.

Materials and Methods

Juvenile seabass with an average weight of 23.6 ± 0.7 g was randomly distributed into 15 tanks (250 L; 5 treatments with 3 replicates of 40 fish each) in a recirculating aquaculture system at CIIMAR facilities. The fish were acclimated for one week and fed a standard commercial diet. After one week of acclimation, fish were hand-fed during four weeks with a control diet (commercial diet) and four experimental diets based on the control and supplemented with two types of bioactive extracts (XAD and Ext3) at inclusion levels of 0.1% and 0.5% (Ext3_0.1%, XAD_0.1%, Ext3_0.5%, XAD_0.5%). The fish were fed daily at 2% of their body weight. Throughout the feeding period fish were maintained at a temperature of $20 \pm 0.5^\circ\text{C}$, a salinity of 32 g/L, and a 12:12 h light: dark photoperiod. At the end of the feeding trial, blood and gut samples were collected ($n = 4$ fish per tank; 12 per treatment), as well as following 72h post-infection. Subsequently, 15 fish per tank were challenged with *P. damsela* subsp. *piscicida* (6×10^4 CFU mL⁻¹). Mortality was monitored daily for 10 days.

Results and Discussion

After four weeks of feeding, no significant differences in zootechnical parameters (weight gain, feed conversion ratio, specific growth rate, survival) were observed, indicating that inclusion of 0.1% and 0.5% of the extracts did not compromise growth performance. While most haematological parameters (i.e., haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration) remained unaltered at 0 h and 72 h post-infection, white blood cell counts were significantly lower in fish fed the Ext3_0.1% diet compared to the control group at 0 h, which also translated in decreased circulating monocytes, lymphocytes and thrombocytes, suggesting an antiinflammatory profile after the feeding period. Following infection, plasma bactericidal activity increased significantly from 0 h to 72 h in fish fed the Ext3_0.5%, XAD_0.1%, and XAD_0.5% diets compared to the control group.

Moreover, Kaplan-Meier survival analysis showed significantly higher survival probability in fish fed at Ext3_0.1% and XAD_0.1%, with approximately twice the survival probability of the control group.

In conclusion, dietary inclusion of *S. ramosissima* bioactives at 0.1% (both Ext3 and XAD) did not affect zoo-technical parameters but significantly increased survival after *P. damsela* subsp. *piscicida* challenge. These results highlight the potential of *S. ramosissima* extracts as functional ingredients for enhancing disease resistance in European seabass aquaculture.

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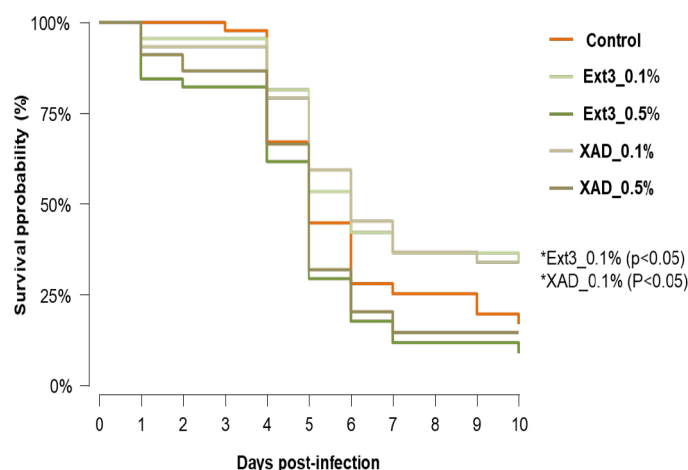


Figure 1: Kaplan–Meier survival curves of European seabass fed experimental diets and challenged with *Photobacterium damsela* subsp. *piscicida* (6×10^4 CFU mL⁻¹). Different curves represent the control and extract-supplemented diets.

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USE OF DESIGN-BASED STEREOLOGY FOR QUANTITATIVE LIVER MICROSTRUCTURAL ASSESSMENT IN A COMMON CARP (*Cyprinus carpio*) FEEDING TRIAL

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Introduction

In the aquafeed industry, the increasing demand for sustainable production has accelerated the development and incorporation of novel feed ingredients and additives, including plant-based proteins and oils. Although these alternatives are promising, their effects on gut and liver overall physiological health must be carefully evaluated before commercial application. Liver histology is routinely conducted in nutritional assays using planar (2-dimensional) sections and morphometry of cellular profiles. In this study, we innovatively applied highly efficient, unbiased (design-based) methods to fish nutrition, using 2-dimensional sectional images to estimate the number and volume of 3-dimensional microscopic structures such as hepatocytes and their nuclei. Today, stereology is frequently used to describe general animal anatomy and in experiments in animal toxicology and ecotoxicology. It is seldom used in fish nutrition, particularly in feeding trials.

Materials and methods

A 96-day nutritional assay was conducted on common carp (*Cyprinus carpio*) with four diets formulated using different ingredients: (1) based on fishmeal (FM); (2) soy protein concentrate (SPC); (3) FM with the addition of linseed oil (FM+LO); (4) SPC with the addition of LO (SPC+LO). All diets also contained maize, wheat and whole soybean. Twelve tanks with 28 fish each (average weight 16.7 (0.1) g) were used for the assay, and each diet was given to fish in three tanks (replicates). At the end of the experiment, two carp per tank were perfused with fixative and their visceral organs harvested. The organs were sectioned using systematic uniform random sampling (SURS) and the selected slabs (1/2 or 1/3) were processed embedded in paraffin (Marcos et al., 2012). Slabs were subsequently sectioned into thick sections (35 μm) and every 10th section was randomly picked for evaluation. The number of hepatocytes in the liver was estimated by superimposing a counting frame of 380.33 μm^2 and a height of 15.00 μm on a motorised stage of an Olympus DP71 (Tokyo, Japan) controlled by the newCAST software (Visiopharm, Denmark). The same software was used for estimating the volume of cells and nuclei by employing the four-way nucleator technique (Marcos et al., 2012). Liver volume was estimated from slabs, while the hepatosomatic index (HSI) was calculated using the volume-to-mass formula (Madureira et al., 2012). Data was analysed by one-way ANOVA followed by the Tukey test when there was a significant group effect.

Results

Liver histology revealed normal microarchitecture across all experimental groups. Hepatocytes were large, with clear cytoplasm and centrally positioned nuclei, and their lipid droplets were prominent. However, hepatic cellularity differed between treatments, with significantly more hepatocytes in the FM-based groups than in the SPC+LO diet ($p < 0.05$). The volume of hepatocytes was also higher in FM-based groups compared to SPC-based groups ($p < 0.05$), while the volume of their nuclei was not statistically different between groups. Estimated liver volume and HSI were highest in the FM group compared to the SPC+LO group ($p < 0.05$).

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Discussion

FM is successfully substituted with SPC in many fish species, and in some of those studies, liver histology was evaluated. Similar to the present study, hepatocytes of rainbow trout (*Oncorhynchus mykiss*) fed exclusively on SPC also showed a reduction in size compared to fish fed FM, but with a significant difference in the appearance of hepatocytes (Escaffre et al., 2007). A common explanation is that the size and appearance of hepatocytes are related to the lipid source in the feed, but interestingly, the addition of LO in the present study did not influence either the appearance or size of the hepatocytes. In pacu (*Piaractus mesopotamicus*) fed with SPC, the same trend was not present, with no difference in hepatocyte size between SPC and FM-fed fish (Ostaszewska et al., 2005). The combination of LO and SPC, which led to a reduction in volume, number of hepatocytes in the liver, and HSI, was not expected, but substitution of FM with SPC in feed for hybrid crucian carp (*Cyprinus carpio* × *Carassius auratus*) can lead to a reduction of antioxidant capacity and non-specific immune function (Zhu et al., 2020). In addition, common carp fed diets based on linseed oil showed an increase in lysozyme activity (Nguyen et al., 2020), and it might be that the combination of these two ingredients can induce negative (possibly even cytotoxic) effects on the liver, but more studies should be conducted before a definite conclusion.

Acknowledgment

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BEYOND MICROALGAE: MAXIMIZING THE PERFORMANCE OF JUVENILE *Ruditapes decussatus* WITH DIETARY SUPPLEMENTS

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Introduction

Supplemented diets have been increasingly investigated in bivalve aquaculture as a strategy to reduce dependence on live microalgae. The production of live microalgae is among the most technically demanding and expensive components of hatchery production. The partial replacement of live microalgae with nutritionally balanced supplements has emerged as a promising strategy to reduce production costs while improving the sustainability of hatchery operations. However, the effectiveness of a supplemented diet depends on several factors, including the biochemical composition, particle size, digestibility and assimilation efficiency. In this context, this study evaluated the effects of a carbohydrate- and lipid-based dietary supplement on juveniles of the grooved carpet shell, *Ruditapes decussatus*.

Materials and Methods

Juvenile clams (*R. decussatus*), with initial shell length of approximately 1 mm, were randomly distributed into 8L-tanks as pooled samples (approximately 0.5 g wet weight, WW, per tank) in a closed system supplied with natural filtered seawater with continuous aeration. Water was renewed every two days, and temperature was maintained at 20 ± 1 °C. Three dietary treatments were tested in triplicate: 100% Microalgae (diet 1), 50% Microalgae (diet 2) and 50% Microalgae supplemented with 25% of a commercial inert diet and 25% of cornstarch (Supplemented diet). Diets consisted of microalgae mix (ratio of 1:1:1), composed of *Tisochrysis lutea*, *Chaetoceros calcitrans*, and *Skeletonema costatum*, administered twice a day at fixed ratios of 10% (100 % Microalgae) or 5% (50 % Microalgae and Supplemented diet) of juveniles WW (g) in algal dry weight (mg). The experimental trial lasted 28 days, with sampling being performed every 7 days. To maintain comparable system density, biomass was standardised at each sampling point. Shell length (mm), daily growth rates (mm day⁻¹; g day⁻¹), organic matter content and biochemical composition were evaluated to address the effects of the experimental diets in juveniles performance. Two-way analysis of variance (ANOVA), followed by Holm-Sidak post-hoc test. Statistical analyses were performed using Sigmaplot 15 statistical package.

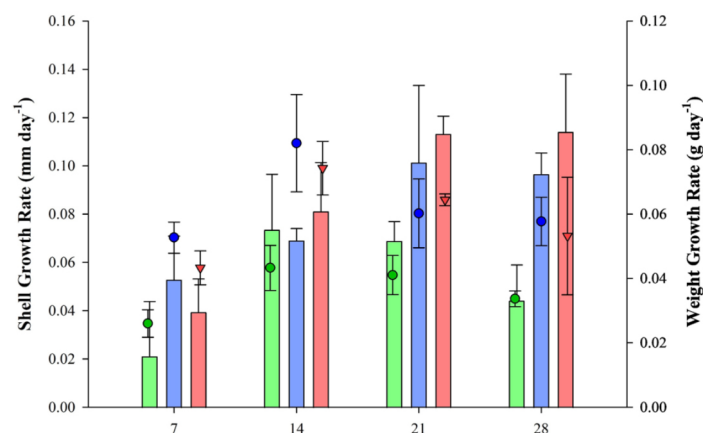


Figure 1 – Shell growth rate (SGR; mm day⁻¹) and weight growth rate (WGR; g day⁻¹) of *Ruditapes decussatus* juveniles fed on different diets: 100% Microalgae; 50% Microalgae and Supplemented diet (50% microalgae supplemented with 25% of a commercial inert diet and 25% of cornstarch).

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Results and Discussion

Throughout the 28-days trial, juveniles fed with 100% Microalgae showed a significantly lower shell length (2.86 ± 0.70 mm, $p < 0.05$). Conversely, juveniles fed with the Supplemented diet exhibited the best shell length (3.84 ± 0.97 mm). During the first 14 days of the trial, growth was mainly reflected in weight increment rather than shell length (fig.1), with 50% Microalgae diet showing a higher increase in grams per day (T14 – 100% Microalgae: 0.043 ± 0.007 g day⁻¹; 50% Microalgae: 0.082 ± 0.015 g day⁻¹; Supplemented diet: 0.074 ± 0.008 g day⁻¹). Afterwards, juveniles exhibited a higher shell length growth rate, with juveniles fed with the Supplemented diet exhibiting higher shell length growth rate (T28 – 100% Microalgae: 0.044 ± 0.015 mm day⁻¹; 50% Microalgae: 0.097 ± 0.009 mm day⁻¹; Supplemented diet: 0.114 ± 0.024 mm day⁻¹). These results are consistent with a shift in energy allocation strategy [1], in which during the first 14 days, juveniles assimilated and allocated energy preferentially to tissue growth, storing reserves in the form of proteins, lipids and carbohydrates, resulting in an increase in biomass (g) and organic matter content. Once an optimal physiological condition was achieved, energy allocation appeared to shift towards shell growth, leading to higher increases in shell length during the second half of the trial.

Although the 100% Microalgae diet provided twice the feeding ratio (10% of WW), juveniles fed on this diet exhibited lower shell and weight growth rates. These results suggest that increasing feeding ratio does not necessarily enhance juvenile growth, supporting the existence of an optimal feeding ratio, above which increases in food availability may reflect physiological constraints on ingestion, digestion and assimilation due to a limited ability of juveniles to efficiently use these reserves [2,3]. Reducing the microalgal ration did not impair juvenile performance, while a partial replacement with an inert supplement further improved shell growth, highlighting the potential of supplemented diets to reduce hatchery costs while maintaining production efficiency.

Acknowledgment

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STRUCTURAL REINFORCEMENT AND BIOCHEMICAL PRIMING: STRATEGIC RESOURCE ALLOCATION IN STARVED GREEN SEA URCHINS *Strongylocentrotus droebachiensis* FED WITH *Saccharina latissima*

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Introduction

In echinoculture, commercial success is often measured by gonad yield and gross biomass. However, these parameters may overlook the critical physiological transitions occurring during nutritional recovery. Green sea urchins (*Strongylocentrotus droebachiensis*) frequently face long periods of food scarcity in the wild, which leads to major metabolic shifts. This study explores the “quality of recovery” by investigating how starved individuals allocate resources from a macroalgal diet (*Saccharina latissima*). The hypothesis was tested that sea urchins prioritize structural stabilization and biochemical “priming” over immediate biomass accumulation.

Materials and Methods

Green sea urchins harvested by hand from Tromsø, Norway, were shipped to Bodø, Norway, and maintained under controlled conditions (7±1 °C) in the laboratory. The animals were divided into two groups: one subjected to long-term starvation and the other fed to satiation with *S. latissima*. Morphological indices, including fresh weight (FW), test diameter, and gonad index (GI), were recorded (n=10). Biochemical analyses of minerals using ICP-OES focused on the tests (shells) and a combined visceral fraction (intestines and coelomic fluid). “True protein” content was determined by summing proteinogenic amino acid residues using HPLC, excluding the non-proteinogenic amino acid taurine to ensure analytical precision. Statistical differences were assessed using independent samples t-tests or Wilcoxon rank-sum tests at a 5% significance level (P = 0.05) in the statistical software package R (v 4.5.1).

Results and Discussion

This study revealed a sophisticated hierarchy in resource allocation. While the starved group demonstrated remarkable structural resilience by maintaining basic body dimensions without significant skeletal degradation, the kelp-fed group exhibited a profound “biochemical reset”.

The fed urchins showed a highly significant increase in the Gonad Index (10.75 ± 2.33% vs. 5.70 ± 2.68%; P < 0.001; Figure 1A). Interestingly, although the total fresh weight did not differ significantly between groups, the internal quality of the tissues underwent a dramatic transformation. In the tests, ash content increased significantly in fed individuals (82.21 ± 3.87 g vs. 79.04 ± 2.70 g; P < 0.05), indicating a strategic investment in skeletal mineralization (Figure 1B). This was accompanied by higher concentrations of matrix-stabilizing amino acids such as glycine and arginine.

In the visceral fraction, a “priming” effect was observed. While the increase in total protein content (141.18 ± 27.50 mg g⁻¹ DW vs. 118.23 ± 23.66 mg g⁻¹ DW) narrowly missed the significance threshold (P = 0.061), specific essential amino acids like phenylalanine, leucine, and methionine increased significantly. This suggests that before significant gross growth occurs, the organism optimizes its metabolic machinery and amino acid pools to support future physiological demands.

Conclusion

Our findings demonstrated that green sea urchins employ a strategic rebuilding process during nutritional recovery. Investment is prioritized toward reinforcing the structural integrity of the test and priming the biochemical capacity of the viscera. For the aquaculture industry, these results suggest that traditional morphological indices should be supplemented with biochemical profiling to fully evaluate the efficacy of “roe enhancement” diets and the physiological health of the animals.

Acknowledgment

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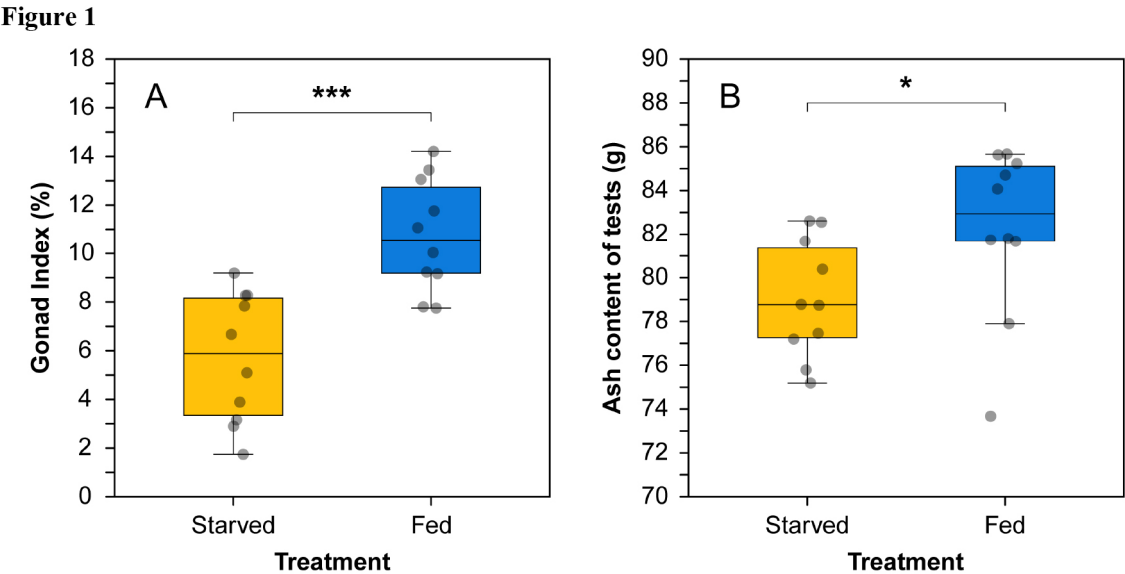


Figure 1: Divergent physiological and structural responses of *Strongylocentrotus droebachiensis* to feeding regime. (A) Gonad Index (%) significantly increased in fed individuals, which illustrates the rapid restoration of energy reserves and reproductive potential upon feeding on *Saccharina latissima*. (B) Ash content (g) of the sea urchin tests significantly increased in the kelp-fed group that reflects a strategic investment of dietary minerals into the skeletal reinforcement. This highlights that nutritional recovery prioritizes internal structural integrity and biochemical priming over immediate increases in gross body dimensions. Data are means and SDs of 10 individual animals per treatment (n = 10). The asterisk above the boxes represents a statistically significant difference (Student's t-test) between “starved” and “fed” animals. Note: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

CANNABIDIOL AND MICROBUBBLE AERATION ENHANCE STRESS RESILIENCE AND PHYSIOLOGICAL HOMEOSTASIS IN EUROPEAN SEA BASS: A MULTI-TISSUE INTEGRATIVE APPROACH

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Introduction

Animal welfare is becoming increasingly important in aquaculture, particularly in intensified production systems. Welfare and stress are closely linked, as reduced stress levels are generally indicative of improved rearing conditions for fish (Balasch & Tort 2019). Among the strategies proposed to improve welfare in aquaculture, environmental enrichment (EE) has shown to provide multiple benefits, including reduced aggression and abnormal behavior, improved growth and survival, lower cortisol, enhanced brain function and cognition (Zhang *et al.*, 2020; Arechavala-Lopez *et al.*, 2022). Similarly, adaptogens have shown potential as effective tools to improve fish welfare. Cannabidiol (CBD), a non-psychoactive compound of *Cannabis sativa*, has been demonstrated to have anti-inflammatory, neuroprotective and anxiolytic properties (Hudson *et al.*, 2019; Mortuza *et al.*, 2023). The present study evaluated the potential effect of dietary CBD supplementation (0.001%) and aeration modification as an occupational EE or, as a novelty, their combination. After 38 days fish were subjected to a confinement stress challenge. Key performance and welfare indicators, such as survival, selected stress and immunological parameters, lipid mediators and the expression of several stress-response and appetite-regulating related genes were analysed in European sea bass (*Dicentrarchus labrax*).

Materials and Methods

Four treatments were evaluated in juvenile of European sea bass over 38 days: control (-CBD/-EE), dietary cannabidiol supplementation (0.001%; +CBD/-EE), EE using a microbubble curtain (-CBD/+EE), and their combination (+CBD/+EE). Growth performance was evaluated followed by a confinement stress challenge with sampling at 2, 24, and 168 h post challenge. Blood samples were collected to determine plasma cortisol and serum lysozyme activity. Head kidney was analysed for lipid mediators using UPLC-MS/MS. The telencephalon, hypothalamus and pituitary gland were collected to evaluate the expression of stress-response related genes (*crf*, *gr2*, *pomc* and *hrt1aβ*) and appetite-regulating related genes (*agrp1*, *agrp2*, *cart2*, *cnr1*, *cnr2*, *npv* and *mc4r*) by RT-qPCR. Survival was also recorded and analysed using Kaplan-Meier survival curve.

Results and Discussion

The utilization of microbubble curtain system as EE improved growth performance and feed efficiency, while also increasing dissolved oxygen levels, whereas dietary CBD had no effect on growth. Conversely, all welfare-promoting strategies increased survival compared with the control group under confinement stress. This result indicates that fish reared under improved environmental or nutritional conditions developed a greater capacity to cope with subsequent stress. Fish exposed to CBD, EE or both showed significantly lower basal plasma cortisol concentrations (ng mL⁻¹) than controls, suggesting an improved physiological state before the stress challenge. Likewise, CBD-fed fish also maintained lower cortisol levels during both acute and chronic stress, indicating rapid anxiolytic effects and improved recovery via modulation of the HPI axis. At the immune stress parameters, CBD supplementation enhanced basal serum lysozyme activity (U mg⁻¹) and promoted a faster post-stress stabilization, suggesting improved innate immunity and a more efficient return to homeostasis in treated fish. Lipid mediator analyses further suggest that these treatments modulated inflammatory regulation. The higher expression of ARA derived lipid mediators in the combined group suggests that CBD and EE may synergistically promote a resolution-oriented inflammatory response, facilitating the clearance of stress-induced damage rather than fostering chronic pro-inflammatory activation. Combined treatment also increased EPA derived lipid mediators often associated with anti-inflammatory pathways. Although their specific physiological roles remain unclear, CBD and environmental enrichment may help to promote a more balanced inflammatory response to stress in European sea bass. The analyses of gene expression in the brain revealed tissue-specific modulation of neuroendocrine pathways related to stress perception, behavioural regulation, and feeding control. In the telencephalon, treatment effects were detected in genes related to stress regulation, cannabinoid and serotonergic signalling, and appetite control. In the hypothalamus,

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several stress-axis genes and appetite-related genes were modulated, indicating that welfare-promoting conditions affected both stress and metabolic control. In the pituitary, the combined treatment downregulated key stress-associated genes. The pattern across brain regions points to a shift toward lower HPI-axis activity and a more stable neuroendocrine state under welfare-promoting conditions in European sea bass. Overall, the present study demonstrates that EE through microbubble-curtain aeration and dietary cannabidiol supplementation can improve stress resilience, physiological homeostasis, and overall rearing performance in juvenile European seabass.

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NURSERY CULTURE PERFORMANCE AND COMPARISON OF GROW-OUT NET TYPES FOR NEW ZEALAND SCALLOP, *Pecten novaezelandiae*, AQUACULTURE

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Introduction

Rising global demand for scallops has led to the collapse of many wild fisheries, increasing the need for sustainable aquaculture. In New Zealand, farming of the native scallop *Pecten novaezelandiae* remains undeveloped, but its emergence will help to address the growing need to diversify the aquaculture industry. Previous farming trials on *P. novaezelandiae* indicated that while suspended culture was technically feasible, the low survival (~ 30%) over the course of the trials, meant it would not be economically viable (Cameron, 1983; Bull et al., 1989). However, these trials involved limited husbandry and did not incorporate key parameters, such as managed stocking densities and routine net cleaning to reduce biofouling, both of which are known to impact scallop performance. This study measured spat performance (growth of shell height and survival) during nursery culture with improved husbandry practices and compared scallop performance during grow-out across a range of net types.

Materials and Methods

Growth and survival of spat was measured during nursery culture. Performance of post-nursery scallops were then compared across three net types (pocket nets, mesh-base lantern nets and plastic-base lantern nets) during the grow-out phase.

Results and Discussion

Of the initial 3600 spat seeded, 89.5% survived the nursery phase (183 days), and 26.2% survived the grow out phase (216 days) resulting in 23.5% survival from seeding through to harvest (399 days). Spat grew from 13.5 mm to 39 mm shell height (SH) over the nursery culture period and then to 70 mm SH over the course of grow-out. In the grow-out phase, pocket nets achieved the highest survival (63.7%) and reached the largest size (79.5 mm SH), followed by mesh-base lantern nets (29.1%, 62.5 mm SH) and plastic-base lantern nets (14.8%, 68 mm SH).

This study confirms that with adequate husbandry practices, performance of *P. novaezelandiae* during nursery, can be improved to levels consistent with international pearl net culture (Widman & Rhodes, 1991). Future research should prioritize optimization of the grow-out stage, which is where lower levels of survival were observed. These results demonstrate that scallops are a potential candidate species to help diversify New Zealand's aquaculture sector. However, further refinement in methodology will be required to improve inefficiencies currently experienced during grow-out.

Acknowledgments

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EFFECT OF THE USE OF A COFEEDING DIET, NATURA PRO, IN TWO DIFFERENT FISH SPECIES OF SCIAENIDAE FAMILY, TOTOABA *Totoaba macdonaldi* AND MEAGRE *Argyrosomus regius*

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Introduction

In 2022, INVE Aquaculture revolutionized the industry with the introduction of Natura range, a premium cofeeding feed lines designed to reduce reliance on rotifers.

The development of the Natura feed line involved extensive research and testing. The feed is designed to be attractive to larvae, with the proper particle size and behaviour in water to encourage capture and ingestion. Be easily digestible and assimilable by the larvae's undeveloped digestive system. To be efficiently convert into energy to support proper growth and survival.

Trials conducted at INVE's research center demonstrated that these feeds could significantly reduce the need for rotifers resulting in a reduction between 50% and 80% for sea bream and completely for sea bass when using the "Green Water Technique; without compromising the growth or survival rates of the larvae.

Following the success in European hatcheries for Sea Bass and Sea Bream, the Natura feed strategy was tested on tropical species such as Barramundi, Yellowtail Amberjack, Snapper with a good success. But, recently in different fish species of Sciaenidae family as Meagre or Totoaba.

Materials and methods

In all the experiments the treatments were Control (following the hatchery protocol with standard concentration of rotifers) versus Natura protocol (developed by INVE to reduce Rotifer consumption using Natura diet).

Data collection and observations were related to Rotifers consumption, survival at first grading, length until first grading, deformity levels and Natura acceptance from first feeding.

In this study, two results will be show, one trial done in Mexico with the specie Totoaba (*Totoaba macdonaldi*) and one trial done in Spain with the specie Meagre (*Argyrosomus regius*).

Results

The results were impressive, showing not only high levels of feed attractivity but also good fry quality with a good growth and better survival rates.

In the trial with Totoaba, different percentages of rotifers substitution were tested (50%, 75%, 100 %) versus the control showing positive results in growth (After 24 dph, LT for "Control" 9 mm, "100%RS" 8.9 mm, "75%RS" 11.2mm, "50%RS" 10.9mm) and in Survival rate (After 26 dph, SR for "Control" 57%, "100%RS" 85%, "75%RS" 75%, "50%RS" 92%). The reduction of rotifers consumption was also obvious.

In the trial with Meagre, a not visible effect on the growth but a positive effect in survival rate (SR Control with 23.6% vs 50%RS with 33.9%). A reduction of 60% of rotifers consumption was observed showing the high interest of this cofeeding diet.

Conclusions

Using Natura feeds in hatcheries worldwide has shown remarkable results in many fish species. Now it is proved too that this cofeeding diet can improve the survival rates and growth, and can mainly reduce the consumption and dependency of rotifers, in species of Sciaenidae family as Totoaba and Meagre. Additionally, the same tendency was observed in another trial done with other fish species of Sciaenidae, Red drum (*Sciaenops ocellatus*).

With this interesting cofeeding diet INVE also reduces the environmental impact of live prey cultivation and helps the farmer in case of issue with the rotifer production. This progress is crucial for securing sustainable food sources for our growing global population and ensuring the long-term success of aquaculture operations.

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Results with Totoaba

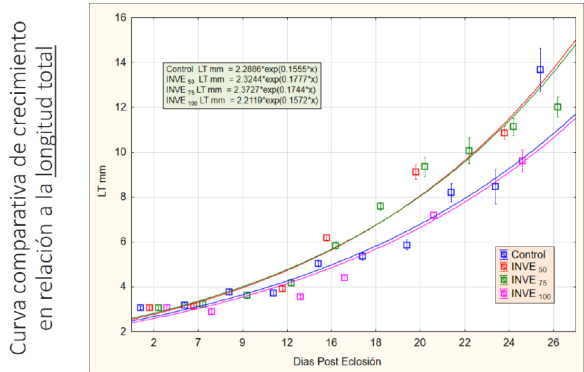


Chart 1: Length at First Grading

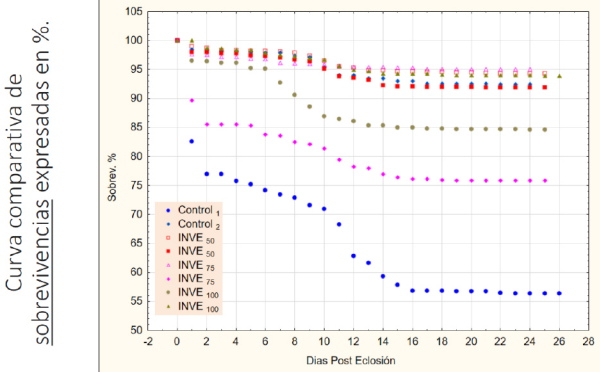


Chart 2: Survival rate

Results with Meagre

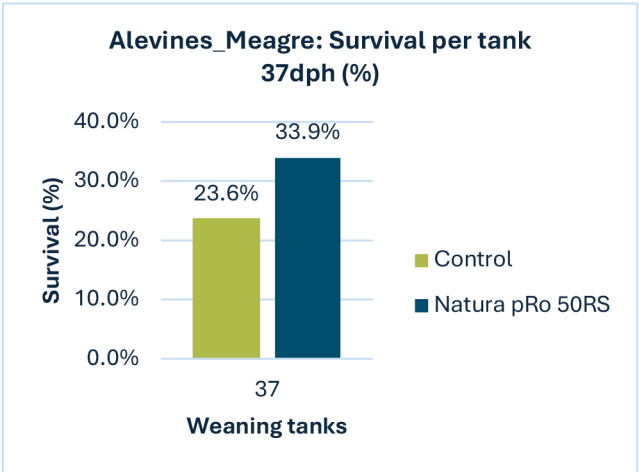


Chart 3: Survival at First Grading

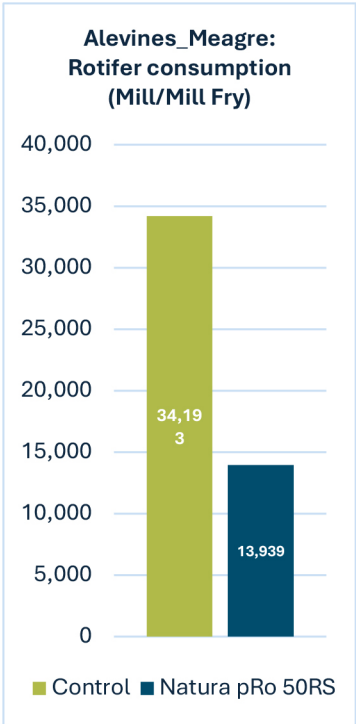


Chart 4: Rotifer Consumption and

Reduction Levels

EPIPHYTIC PHYTOPLANKTON ON ROCK SUBSTRATES IN CLAM TANKS: EFFECT OF TANK AGE

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Introduction

Rock substrates placed on the bottom of bivalve aquaculture grow-out tanks may act as artificial surfaces for the development of epiphytic biofilms, which are progressively colonized by benthic microalgae, particularly diatoms. Similar interactions between bivalves and diatom-dominated biofilms have been reported in mussel-associated habitats, where shellfish structures influence diatom community composition and biofilm functioning (Andriana et al., 2021). Periphyton is a diverse benthic community typically dominated by phytoplankton groups such as diatoms, green algae and cyanobacteria (Lamprecht et al., 2022), and this biofilm may influence water quality, oxygen dynamics and the trophic environment available to cultured clams, while also representing a recognized biofouling and management issue in aquaculture facilities (Wu et al., 2026). The influence of tank age on the taxonomic composition and richness of epiphytic phytoplankton communities remains poorly understood in land-based clam farming systems. This study provides a descriptive comparison of epiphytic phytoplankton assemblages colonizing rock substrates from two clam grow-out tanks with contrasting operational ages, neither of which had been emptied or cleaned since being put into operation.

Materials and Methods

Rock substrates were sampled from two clam grow-out tanks at Delta Futuro Soc. Agr., a land-based, closed-circuit clam farming facility located in the Po River Delta lagoon area (Sacca di Goro), Italy: Tank 03, in operation for approximately 24 months and never emptied or cleaned, and Tank SN14, in operation for approximately 8 months and never emptied or cleaned. Epiphytic phytoplankton was removed from the rock surfaces with a toothbrush following the standard methods BS EN 13946:2003/BS 6068-5.33:2003 (CEN, 2003), and samples were preserved in 4% formalin. Microscopic analyses were performed using a light microscope under 400x magnification, allowing the observation of morphological features required for taxonomic identification. Taxa were identified to the lowest possible taxonomic level (genus or species) using standard taxonomic keys, and presence/absence of each taxon was recorded for each tank.

Results and Discussion

A total of 31 epiphytic taxa distributed across five phyla were identified on the tank rock substrates: Bacillariophyta (20 taxa), Cyanobacteriophyta (6), Chlorophyta (2), Euglenophyta (2) and Dinoflagellata (1). Diatoms were the dominant group in both tanks, accounting for approximately two thirds of the taxa recorded, consistent with the typical dominance of diatoms in periphytic biofilms on submerged substrates (Miao et al., 2021). Overall taxonomic richness was higher in Tank SN14 (29 taxa, 94% of the total pool) than in Tank 03 (21 taxa, 68%), despite Tank SN14 being much younger and having accumulated biofilm for a much shorter time (8 months versus 24 months without being emptied or cleaned). Taxa shared by both tanks included common biofouling diatoms such as *Cylindrotheca closterium*, *Planorhynchus* sp., *Navicula* sp., *Gyrodinium aureolum*, *Pleurosigma intermedium*, *Pinnularia* sp., *Amphora ovalis*, *Synedra* sp., *Ulnaria ulna*, *Licmophora* spp. and *Melosira varians*, indicating that these are early and persistent colonizers regardless of biofilm age. Taxa found exclusively in Tank SN14 (younger biofilm) included *Tryblionella* sp., *Encyonema* sp., *Sellaphora* sp., *Stenopterobia sigmatella*, *Climacosphecia moniligera*, *Chlamydomonas reinhardtii*, *Raphidiopsis raciborskii*, *Parvodinium umbonatum* and *Trachelomonas volvocina*. In contrast, only *Eunotia* sp. and *Gyrodinium aureolum* were exclusive to the older biofilm of Tank 03. This pattern suggests that the younger biofilm (8 months) was still in an early, opportunistic colonization stage, supporting a wider range of pioneer diatom, cyanobacterial, and flagellate taxa, whereas the long-standing biofilm of Tank 03 (24 months) may have experienced increasing competitive interactions and self-shading as biofilm biomass and thickness increased during maturation, resulting in the dominance of fewer, more persistent taxa (Zhang et al., 2023). These results suggest that epiphytic phytoplankton richness does not necessarily increase continuously during biofilm development, but instead reflects changes in community composition and species replacement during ecological succession,

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with early stages supporting more diverse taxa and later stages becoming dominated by fewer persistent taxa. However, this interpretation is preliminary and requires validation with more experimental units and temporal sampling. In terms of taxonomic composition, Bacillariophyta accounted for 20 of the 31 taxa recorded (14 in Tank 03 and 18 in Tank SN14), followed by Cyanobacteriophyta with 6 taxa (5 in Tank 03 and 6 in Tank SN14), Chlorophyta and Euglenophyta with 2 taxa each (1 in Tank 03 and 2 in Tank SN14 for both phyla), and Dinoflagellata with a single taxon, present only in Tank SN14. This distribution confirms diatoms as the dominant phytoplankton group in both tanks, followed by cyanobacteria, with green algae, euglenoids and dinoflagellates contributing marginally to overall richness.

Conclusion

Contrary to expectations, epiphytic phytoplankton richness was higher in the younger tank than in the older one, indicating that richness reflects the degree of biofilm maturation, with younger biofilms supporting a wider range of pioneer taxa and older biofilms converging toward a more selected, diatom-dominated assemblage. Since clams graze directly on epiphytic biofilms as a supplementary food source, the higher richness observed in younger tanks suggests that early-stage biofilms may provide more diverse and nutritionally valuable food resources, potentially enriching the trophic environment and supporting clam production in land-based aquaculture systems.

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SODIUM NITRATE DEPRIVATION AS THE PRIMARY INDUCER OF ASTAXANTHIN AND CAROTENOID BIOSYNTHESIS IN *Haematococcus pluvialis*

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Introduction

Haematococcus pluvialis (Chlorophyta) is the main biological source of astaxanthin, a xanthophyll carotenoid with potent antioxidant activity and broad applications in the nutraceutical, aquaculture and cosmetic industries (Oslan et al., 2021). Under nutrient-deprivation stress, particularly nitrogen (NaNO_3), vegetative cells cease division and encyst, accumulating astaxanthin in cytoplasmic lipid droplets. Although the inductive effect of nitrogen deprivation is well documented, few studies statistically quantify it together with phosphorus (KH_2PO_4) availability across a wide concentration range, including total N deprivation as a formal design point (Ren et al., 2021; Rizzo et al., 2022). This study applied a central composite rotatable design (CCRD) and response surface methodology (RSM) to evaluate the effects of NaNO_3 and KH_2PO_4 on astaxanthin, total carotenoids and chlorophyll a production by *H. pluvialis*, with experimental validation of the optimal condition.

Materials and Methods

A CCRD 2^2 ($\alpha = 1.41$; three central-point replicates; $n = 11$ runs) was built for NaNO_3 and KH_2PO_4 concentrations, with the lower axial point of NaNO_3 ($x_1 = -1.41$) corresponding to total nitrogen deprivation (0 g/L). *H. pluvialis* was cultivated in 250 mL Erlenmeyer flasks (100 mL working volume) in modified Bold's Basal Medium (BBM) at $22 \pm 1^\circ\text{C}$, 100 $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$ (16:8 h light:dark) for 21 days. Chlorophyll a and total carotenoids were determined spectrophotometrically using the equations proposed by Lichtenthaler (1987), and astaxanthin was quantified indirectly by absorbance at 492 nm. Full second-order quadratic models were fitted by OLS (R v4.2.3, rsm package), and the isolated effect of NaNO_3 deprivation was assessed by fold change, Z-score and Pearson correlation. The optimal condition was validated with three independent replicates.

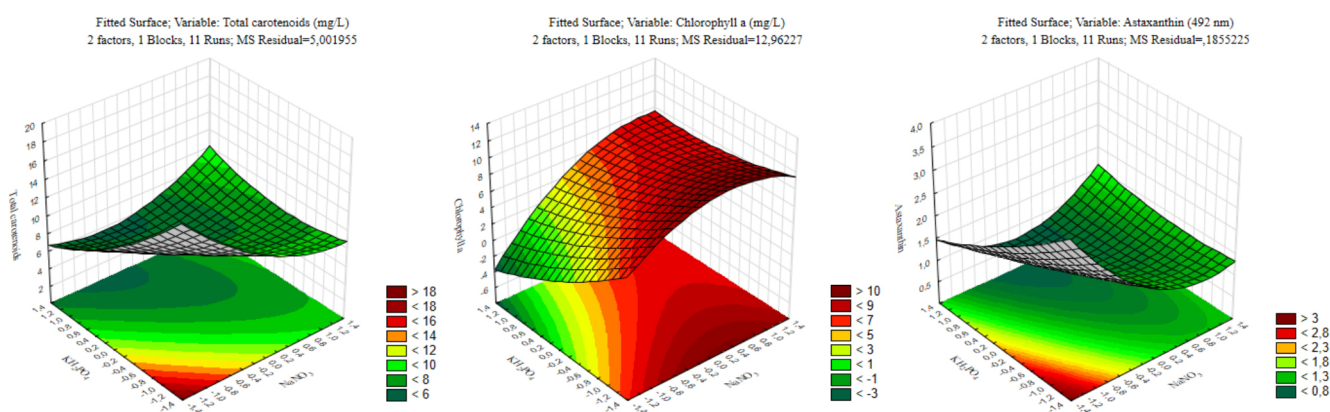


Figure 1. Three-dimensional response surfaces of the RSM quadratic model (CCRD 2^2 , $\alpha = 1.41$; 2 factors, 11 runs) for total carotenoids (left; MS Residual = 5.002), chlorophyll a (center; MS Residual = 12.962), and astaxanthin at 492 nm (right; MS Residual = 0.186) in *Haematococcus pluvialis*. Axes represent coded variables: NaNO_3 (x_1) and KH_2PO_4 (x_2), ranging from -1.41 to $+1.41$. Colour scale indicates predicted response intensity (red = high; green = low).

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Results and Discussion

Total NaNO_3 deprivation (Run 5; $[\text{NaNO}_3] = 0 \text{ g/L}$; $[\text{KH}_2\text{PO}_4] = 8.75 \text{ g/L}$) induced the highest astaxanthin production of the design (ABS 492 nm = 2.823), a 2.38-fold increase over the mean of the remaining runs (1.19 ± 0.42 ; Z-score = 3.89σ), and 1.67-fold higher total carotenoids (13.57 vs. $8.15 \pm 2.62 \text{ mg/L}$), while chlorophyll a declined 0.17 times the mean ($1.14 \text{ vs. } 6.91 \text{ mg/L}$), reflecting the antagonism between chlorophyll synthesis and secondary carotenoid accumulation under nitrogen stress. The Pearson correlation between $[\text{NaNO}_3]$ and astaxanthin was negative but not statistically significant ($r = -0.506$; $p = 0.113$), indicating a tendency toward higher astaxanthin accumulation at lower NaNO_3 concentrations. The RSM quadratic model for astaxanthin yielded $R^2 = 0.769$ ($R^2_{\text{adj}} = 0.539$), with the linear ($\beta_1 = -0.3589$; $p = 0.065$) and quadratic ($\beta_{11} = +0.4156$; $p = 0.071$) NaNO_3 terms marginally significant. Response surface analysis (Figure 1) confirmed that the astaxanthin production gradient is dominated by the NaNO_3 axis, increasing monotonically toward total nitrogen deprivation at the domain boundary. KH_2PO_4 and the $\text{NaNO}_3 \times \text{KH}_2\text{PO}_4$ interaction were not significant, indicating approximately additive effects within the studied range. Model validation at the optimal point yielded mean observed values of 2.068 ± 0.057 ABS 492 nm for astaxanthin, $10.644 \pm 0.333 \text{ mg/L}$ for total carotenoids, and $0.582 \pm 0.052 \text{ mg/L}$ for chlorophyll a, with relative errors of 11.7%, 6.8% and 6.4% respectively, confirming adequate model predictive capacity. These results support two-stage cultivation strategies (vegetative growth in complete medium followed by NaNO_3 -free induction) as an effective approach for maximizing astaxanthin production by *H. pluvialis* in bioprocesses.

Conclusion

Total NaNO_3 deprivation was the dominant driver of astaxanthin and total carotenoid accumulation in *H. pluvialis*, whereas KH_2PO_4 and the $\text{NaNO}_3 \times \text{KH}_2\text{PO}_4$ interaction played only a marginal, additive role within the tested range. The RSM quadratic model captured this trend with adequate predictive capacity, as confirmed by experimental validation at the optimal point (relative errors below 12% for all three pigments). Together, these results indicate that abrupt, complete nitrogen starvation, rather than moderate nitrogen limitation, is the most effective single trigger of the green-to-red transition, supporting two-stage cultivation as a practical strategy for maximizing astaxanthin yield in *H. pluvialis* bioprocesses.

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OPTIMIZING THE WEANING OF THE AFRICAN CATFISH (*Clarias gariepinus*) FRY FROM DECAPSULATED ARTEMIA TO COMMERCIAL AQUACULTURE FEED

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Introduction

Early larval rearing of african catfish (*Clarias gariepinus*, Burchell 1822) remains a critical challenge, as newly hatched larvae possess an immature digestive system with limited proteolytic activity, restricting efficient utilization of dry feeds. Decapsulated *Artemia salina* cysts are therefore widely used as first feed before progressive transition to commercial starter diets. Thus optimizing this weaning transition is essential to maximize survival and growth while reducing *Artemia* dependency.

Materials and methods

Two experiments were carried out independently in the recirculating aquaculture system of the Department of Aquaculture at the Hungarian University of Agricultural and Life Sciences consisting of 25 rearing tanks. (water temperature $25 \pm 1^\circ\text{C}$). For both experiments a total of five treatments were tested in five replicates per treatment. African catfish larvae were initially fed with decapsulated *Artemia salina* cysts, followed by gradual replacement with a commercial starter diet (ALLER INFA EX GR). In the first trial, the effect of the timing of commercial feed introduction was evaluated. The duration of the weaning was 3 days in all treatments. Commercial feed was introduced at different times (0, 3, 6 and 9 days) after the start of exogenous feeding (4 dph). Five treatments were set: control (100 % artemia), 0+3, 3+3, 6+3, and 9+3 days. In the second trial, the effect of the duration of the weaning period was assessed. Based on the results of the first experiment the introduction of the commercial feed started on the third day. Growth performance was assessed by measuring wet body weight. Survival rate was calculated as the percentage of fish remaining at the end of the trial. Data were analyzed using one-way ANOVA ($\alpha < 0.05$).

Results

In experiment 1, the 0+3 and 3+3 treatments showed the highest survival rates, compared the 9+3 treatment and the control group. In contrast, the 9+3 treatment had the highest final weight, while the 0+3 and 3+3 groups showed the lowest final weight, yet the other treatments showed no notable differences. In experiment 2, the control showed the highest final weight while 1 and 7 days weaning group showed the lowest. No significant difference was detected among the 3 and 5 days groups for final weight, although the three short weaning periods (1, 3 and 5 days) resulted in higher survival than the 7 days group or the control. (Table 1.)

Table 1: The final weight and survival rate of *Clarias gariepinus* weaned to commercial diets at 0-9 days for a weaning period of 3 days (experiment 1) and different weaning durations (experiment 2).

Experiment	Treatments	Final weight in mg	survival rate %
1	0+3 days	11.69 ± 1.8^b	57.76 ± 7.9^a
	3+3 days	13.8 ± 2.3^b	63.64 ± 4.3^a
	6+3 days	18.25 ± 4.6^{ab}	40.28 ± 2.8^b
	9+3 days	19.64 ± 4.5^a	31.32 ± 1.2^c
	Control	16.48 ± 2.7^{ab}	31.24 ± 4.1^c
2	1 day	10.81 ± 1.1^b	60 ± 3.5^a
	3 days	12.38 ± 2.0^{ab}	58.48 ± 4.3^a
	5 days	11.5 ± 1.3^{ab}	52.08 ± 2.5^{ab}
	7 days	11.05 ± 0.7^b	48.52 ± 6.5^b
	Control	13.8 ± 1.7^a	45.6 ± 3.6^b

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Discussion

The results demonstrate that both the *Artemia salina* feeding duration and weaning transition length affect significantly larval growth and survival in *C. gariepinus*. The 3+3 treatment showed the best overall performance in Experiment 1 (13.8 ± 2.3 mg; $63.64 \pm 4.3\%$ survival), suggesting an optimal weaning period consistent with Verreth & van Tongeren (1989). In Experiment 2, short weaning periods (1-5 days) outperformed longer ones, thus supporting the importance of a gradual but quick dietary transition, as reported by Cherpikui-Boit *et al.*, (2011). The underperformance of the 7 days treatment suggests that prolonged transitional feeding delays digestive adaptation without the continued nutritional benefit of *Artemia*.

Conclusion

Overall, a 3 days *Artemia* prefeeding period combined with a 3 days progressive weaning schedule appears as the most effective protocol for *C. gariepinus* larval rearing. Future work integrating digestive enzyme assays, intestinal histology, and gut microbiome characterization will be essential to explain the mechanisms underlying these results.

Acknowledgment

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TO BE IN A LAKE OR A RIVER: ECOTYPE DIVERGENCE IN BROWN TROUT

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Introduction

Brown trout (*Salmo trutta*) is known for its life-history variation, including stream and lake ecotypes that differ in growth, pigmentation, maturation and behavior. The genomic basis of this differentiation remains poorly understood, particularly for lake ecotypes (Schöffmann, 2021). It is unclear whether similar phenotypes across geographically distant systems reflect shared ancestry, repeated local adaptation, or population-specific evolutionary trajectories (Collins et al., 2022; Moran et al., 2024) but some species such as *Oncorhynchus mykiss* demonstrate variation in life history strategies with some individuals remaining exclusively resident in freshwater, whereas others undergo anadromous migration. Because there is limited understanding of genes involved in this life history variation across populations of this species, we evaluated the genomic difference between known anadromous (*n* = 39). Here, we use a cost-effective whole-genome sequencing approach to investigate genomic differentiation between lake and stream ecotypes across four independent European population pairs, aiming to identify regions repeatedly associated with ecotype divergence (Lou et al., 2021) "given": "Arne"}, {"family": "Wilder", "given": "Aryn P."}, {"family": "Therkildsen", "given": "Nina Overgaard"}], "issued": {"date-parts": [{"2021", 12, 31}]}}, {"schema": "https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} .

Materials and Methods

A total of 116 individuals from four lake–stream population pairs were analysed: Lake Bled–Sava Bohinjka and Lake Bohinj–Ribnica (Slovenia), Lake Siljan–Fjätälven (Sweden), and Lake Lucerne–Scheidgraben (Switzerland). Using low-coverage whole-genome sequencing, we assessed population structure with PCA and ancestry-based methods. Loci under selection between ecotypes were identified using PCANGSD, while a complementary Bayesian genotype–phenotype association model implemented in BAYPASS was used to detect loci consistently differentiated across population pairs (Foll & Gaggiotti, 2008; Korneliussen et al., 2014) we demonstrate that it is possible to avoid them by carefully choosing the populations that should be included in the analysis. We analyze two previously published data sets: a human data set of codominant markers and a *Littorina saxatilis* data set of dominant markers. We also perform a detailed sensitivity study to compare the power of the method using amplified fragment length polymorphism (AFLP). Candidate regions were functionally annotated and evaluated for gene ontology enrichment.

Results

Genome-wide analyses revealed clear differentiation among geographic systems, while lake–stream divergence varied among population pairs. Selection scans identified multiple genomic regions associated with ecotype differentiation, with BAYPASS detecting 49 candidate regions under the strongest evidence of divergent selection. Most signals were narrow and method-specific, suggesting a polygenic basis of divergence rather than control by a single major-effect region.

Analysis of Tajima's D across candidate regions indicated deviations from neutrality between ecotypes, consistent with selection and/or demographic differences. Several candidate regions were associated with genes involved in cell membrane structure, ion transport and intercellular adhesion, suggesting that interactions with the external environment and tissue organization may play a role in ecotype divergence.

Discussion

Our results confirm genomic differentiation between lake and stream ecotypes of brown trout but show that this divergence is not consistent across all systems. The presence of candidate regions across independent population pairs suggests some shared genomic responses, while limited overlap among methods and populations highlights the importance of local demographic history and population-specific adaptation.

Functional annotation indicated enrichment of genes related to cell membrane structure, ion transport and intercellular adhesion, pointing to a potential role of environmental interaction and tissue-level processes in ecotype divergence. These functions are consistent with adaptation to contrasting lake and river habitats, which differ in hydrodynamics, resource availability and physicochemical conditions.

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More broadly, this study demonstrates that low-coverage whole-genome sequencing, combined with genotype-likelihood-based approaches, provides a robust framework for detecting adaptive divergence in non-model salmonids. These findings contribute to understanding the genomic basis of life-history variation and may inform conservation and management of locally adapted brown trout populations.

Acknowledgment

This work was conducted as part of doctoral research at the University of Ljubljana, Biotechnical Faculty, with support from collaborating fisheries organizations and international partners who provided samples from Slovenia, Sweden and Switzerland.

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TRENDS IN AQUACULTURE FINANCING: EVIDENCE FROM RURAL CREDIT IN BRAZIL

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Introduction

Brazil has strong potential for aquaculture development, supported by its extensive coastline, vast hydrographic basins, and abundance of freshwater and land resources. In addition, favorable climatic conditions and the natural occurrence of species with zootechnical and commercial relevance reinforce the conditions for sectoral expansion.

Nevertheless, national aquaculture production remains far below its potential. Among the main challenges limiting sector growth is restricted access to credit lines, a key factor for the expansion and modernization of the activity. Brazilian literature indicates that rural credit available for the fishing and aquaculture chains remains insufficient in relation to their needs and sectoral importance.

Although fish farming is highly important, scientific studies still present gaps regarding the socioeconomic factors that affect its development. Research has predominantly focused on technological and biological aspects of species, while environmental, economic, and financial issues have received less attention, limiting a comprehensive understanding of the sector's challenges.

In this context, the analysis of rural credit becomes particularly relevant, although it remains an underexplored field within management studies. Therefore, the present research aims to investigate the recent trajectory of rural credit allocation for aquaculture in Brazil and its associated challenges.

Methodology and Data Sources

The study employs a descriptive and quantitative approach, enabling the analysis of both the statistical evolution of rural credit and the institutional context of aquaculture financing. The quantitative component relies on secondary data from the Central Bank of Brazil's Rural Credit Data Matrix, covering the period from 2019 to 2024, with information on the number of contracts and the total volume of credit allocated to aquaculture, disaggregated by purpose (operating/costing, investment, and commercialization).

To evaluate whether credit expansion has kept pace with productive growth, the study incorporates production data from the Brazilian Institute of Geography and Statistics (IBGE). The analysis is further complemented by sectoral data on production and exports from FISH Brazil and FAO (Food and Agriculture Organization), as well as official documents such as the 2024/2025 Agricultural Plan, which were used to identify available credit lines for the sector.

Results and Discussion

The empirical analysis reveals a significant expansion of rural credit directed to aquaculture between 2019 and 2024. Total credit volume increased from USD 95.6 million in 2019 to USD 235.2 million in 2024, representing an accumulated growth of approximately 147% over the period. This upward trajectory demonstrates a strong expansion in financial resources allocated to the sector.

A striking feature of the credit composition is the overwhelming predominance of operating (costing) credit. On average, 90% of total financial volume was allocated to operations, while investment credit accounted for approximately 10%, and commercialization credit represented a negligible share. This distribution indicates that financing has primarily supported short-term production cycles rather than long-term structural expansion and modernization.

Between 2020 and 2023, the average annual growth rate of rural credit for aquaculture reached 21.8%, while the average annual growth rate of national production value was 18.4%. In certain years, such as 2022 and 2023, credit growth even surpassed production growth.

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The study identifies a strong regional concentration of credit and production in the South and Southeast regions, where infrastructure, logistics, technological adoption, and integration with industrial chains are more advanced. Cooperative systems and vertically integrated agribusiness groups—some originally specialized in poultry and pork—have entered tilapia production, enhancing efficiency and market access.

In contrast, the North and Northeast regions face greater obstacles, including land tenure irregularities, environmental licensing difficulties, limited technical assistance, and weaker institutional integration. In states such as Pará and Amapá, small-scale producers often lack the documentation and guarantees required to access formal credit, which exacerbates regional disparities.

Despite encouraging financial indicators, several bottlenecks constrain broader credit expansion: (I) Excessive bureaucracy in credit approval procedures; (II) Complex and costly environmental licensing processes; (III) Land tenure irregularities and difficulties in obtaining water-use permits; (IV) High interest rates in certain modalities; (V) Inadequate infrastructure in emerging production regions; (VI) Credit lines not fully adapted to aquaculture production cycles (e.g., short repayment terms for operating credit); (VII) Limited use of private financial instruments such as Agribusiness Credit Bills (LCA), Agribusiness Receivables Certificates (CRA), and agribusiness investment funds (Fiagros).

The requirement for complete documentation—including proof of land ownership, environmental compliance, and financial regularity—frequently prevents small and medium producers from accessing financing. Studies cited in the research indicate that, in some cases, the payment guarantee structure of existing credit lines is incompatible with the biological and commercial cycles of fish farming.

Conclusions

The study concludes that rural credit for aquaculture in Brazil has experienced robust growth between 2019 and 2024, both in financial volume and in the number of contracts, which contradicts the existing literature. The expansion of credit has broadly accompanied the increase in national production value, signaling positive momentum for the sector.

However, persistent structural and institutional constraints continue to limit more inclusive and geographically balanced development. Bureaucracy, environmental and land regularization challenges, infrastructural deficiencies, and regional concentration remain significant barriers.

To unlock Brazil's full aquaculture potential, public policies must move beyond mere budget expansion and focus on institutional reforms, credit adequacy, regulatory simplification, and regional equity. By addressing these structural issues, rural credit can more effectively contribute to sustainable economic growth, income generation, and social inclusion within Brazil's expanding aquaculture chain.

DIETARY 1.3-1.6 YEAST β -GLUCANS ENHANCE IMMUNE RESPONSE AND DISEASE RESILIENCE IN EUROPEAN SEABASS CHALLENGED WITH *Tenacibaculum maritimum*

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Introduction

Aquaculture is one of the fastest-growing food-producing sectors worldwide, but its expansion is heavily constrained by infectious diseases. Preventive approaches that strengthen host defenses are essential, and particularly against *Tenacibaculum maritimum* infection, a major threat to marine finfish aquaculture. Functional feeds incorporating bioactive additives such as β -glucans have shown interesting results in terms of fish immunity modulation and pathogen resilience. However, the capacity of β -glucans to coordinate mucosal and systemic responses during a *T. maritimum* infection remains underexplored. This study aims to investigate the immunomodulatory effects of dietary β -glucans in European seabass and following a *T. maritimum* challenge, assessing both mucosal and systemic immune responses.

Materials and Methods

European seabass (*Dicentrarchus labrax*) juveniles, having an initial mean body weight of 11.3 g were randomly distributed among 24 tanks (8 tanks per dietary treatment) and were fed with a diet containing 0, 0.06 or 0.12% of purified 1.3-1.6 yeast β -glucans (BG). After 4 weeks of feeding, 4 replicated tanks per dietary treatment were subjected to a *Tenacibaculum maritimum* infection by bath inoculation while the other 4 tanks per treatment were used as non-infected control. Zootechnical parameters were monitored during the first 4 weeks of feeding. Mortality was recorded throughout the experiment. At 24h and 7 days post-infection, fish immune response was evaluated through mRNA expression levels of immune-related genes in skin and posterior intestine. Those analyses were complemented by measurement of some plasma immune-related parameters, including specific IgM against *T. maritimum* at 3 weeks following infection.

Results and Discussion

Growth performance and feed utilization were not affected by dietary BG supplementation. However, BG modulated systemic and mucosal immune responses following *T. maritimum* infection. At 24 h post-infection, the 0.12% BG diet maintained circulating white blood cell levels ($p < 0.05$) and increased plasma lysozyme activity in infected fish ($p < 0.05$), indicating enhanced early immune readiness. Specific IgM against *T. maritimum* also increased in both BG-fed fish groups 3 weeks after challenge ($p < 0.05$), suggesting enhanced adaptive immunity. In the skin, the 0.12% BG diet promoted an early upregulation of pro-inflammatory and antimicrobial-related genes at 24h post-infection ($p < 0.05$), followed by an increased expression of regulatory cytokines (*il10* and *tgfb*) at 7 days post-infection ($p < 0.05$), indicating a balanced immune response. Multivariate analysis highlighted the skin as the primary responsive interface. In contrast, intestinal immune regulation was more controlled, with *il10* expression reduced in infected fish fed the 0.12% BG diet at 7 days post-infection ($p < 0.05$).

Conclusion

These results demonstrate that dietary yeast BG can enhance localized (in skin) and systemic immunity in European seabass, in the context of a *T. maritimum* challenge, without compromising growth. The highest dose tested, 0.12% BG, was the one inducing the most significant enhancements in disease resilience.

Acknowledgment

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MULTI-STRESSOR DRIVERS OF MASS MORTALITY IN MEDITERRANEAN MUSSEL (*Mytilus galloprovincialis*) AQUACULTURE: A CASE STUDY FROM THE NORTHERN ADRIATIC

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Introduction

Mass mortality events affecting the Mediterranean mussel *Mytilus galloprovincialis* have been reported with increasing frequency across the Mediterranean basin. In the Slovenian Sea, mussel farmers have recorded near-total ($\approx 100\%$) mortality over two consecutive production seasons, representing a critical threat to local aquaculture. Within the TEAMFORADRI project (Transnational mussEl preservation systeMs FOR the ADRIatic Sea; Interreg IPA ADRION 2021–2027), a monitoring programme was established to characterise mussel health dynamics and identify the drivers of mortality, combining field observations, biometric and biomechanical analyses, histopathological examination, and species identification.

Materials and Methods

Monthly sampling was conducted from June 2025 to May 2026 (12 events) at a mussel farm in the Bay of Piran, collecting 50 farmed and 30 wild *M. galloprovincialis* per event ($n = 960$ in total). Visual inspection of mussel lines was carried out, recording the presence of biofouling organisms. Biometric measurements (shell length, width, height, wet and dry tissue and shell weights) were recorded. Byssus thread tensile strength was measured using a force gauge (Babarro and Comeau, 2014), and the Condition Index ($CI = \text{dry viscera weight} / \text{shell volume (g L}^{-1}\text{)}$) was calculated as an indicator of physiological state (Lobel et al., 1991). A subset of samples (30 farmed and 30 wild per event) was submitted for histopathological analysis of gills, digestive gland, gonad, mantle, musculature, foot, heart and kidney. The presence of epifauna and flatworm species were recorded.

Results

Biometrics and field observations: CI in farmed mussels showed a progressive seasonal decline from a median of 20.3 g L^{-1} in July 2025 to a winter minimum of 9.7 g L^{-1} in February 2026, with a partial spring recovery to 14.8 g L^{-1} in March 2026. Byssus thread tensile strength followed a similar pattern, with the lowest value in July 2025 ($7.8 \pm 3.2 \text{ N}$, $n = 20$) and the highest in November 2025 ($12.7 \pm 4.5 \text{ N}$, $n = 20$). Field inspections consistently recorded the presence of biofouling on mussel lines throughout the monitoring period.

Histopathology: The endoparasitic copepod *Mytilicola intestinalis* was the most prevalent finding, detected in 75% of farmed individuals in June and 40% in July 2025. *Rickettsia*-like intracellular bacteria were observed in the gill epithelium without a marked inflammatory response (20% farmed, 40% wild in June; 30% farmed, 55% wild in July). Focal granulomas with haemocyte infiltration, areas of tissue necrosis in the mantle and musculature, and ciliates in the digestive gland epithelium were recorded in both sampling events and both groups. Suspected myocarditis, characterised by haemocyte infiltration surrounding cardiomyocytes was identified in 15% of individuals in the July 2025 sampling, in both farmed and wild mussels. The turbellarian *Urastoma cyprinae* was detected in gill tissue sections from July 2025 onwards (15% farmed, 15% wild).

Associated fauna: Biofouling species were recorded along the hanging ropes in the mussel farm. A predatory polyclad flatworm native to the Pacific Ocean *Postenterogonia orbicularis*, was present during the whole period of present study.

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Discussion

These results indicate a complex, multi-stressor aetiology for the observed mass mortality rather than a single causative agent. The presence of the predatory flatworm *Postenterogonia orbicularis*, native to the Pacific Ocean was confirmed in all investigated samples. The species were recorded for the first time in a mussel farm in October 2023 recognised as a voracious predator, but the impact on economic loss was not quantified (Ramšak et al., 2024). The concurrent seasonal decline in CI and byssus thread strength, reaching minimum values in summer and mid-winter respectively, together with a high prevalence of *Mytilicola intestinalis*, *Rickettsia*-like bacteria, and haemocytic inflammatory responses observed in histopathological analyses, suggests that cumulative physiological stress reduces host resilience and may amplify the impact of pathogens. Field observations further indicate that biofouling species differ markedly between upper and lower sections of culture ropes, and the potential presence of the tunicate *Clavellina sp.* and other biofouling species requires further investigation.

A temperature difference of approximately 5 °C recorded between the sea surface (measured at 2 m depth) and the bottom layer at 10 m depth at the study site suggests a practical approach for mitigating the identified stressors. Evidence shows that lower water temperatures reduce trade-offs between byssus function and reproductive investment, maintaining stronger attachment capacity during thermally stressful seasons and decreasing overall mortality risk (Mizuta and Wikfors, 2019). Based on this, the project proposes piloting an underwater cultivation structure positioned at 10 m depth, using vertical cultivation lines to access cooler bottom layers. The infrastructure, developed as an Integrated Multi-trophic Aquaculture (IMTA) approach, would allow direct comparison of mussel health metrics between conventional surface farms and depth-managed systems as an adaptive aquaculture strategy.

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TRACKING PERSISTENCE AND SPREAD OF *Neoparamoeba perurans* USING DUAL HOST-SYMBIONT AMPSEQ GENOTYPING

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Introduction

Amoebic Gill Disease (AGD) is one of the most significant health constraints affecting Atlantic salmon aquaculture caused by the marine amoeba *Neoparamoeba perurans*. Despite its economic importance, tools for high-resolution epidemiological surveillance remain limited, restricting the ability to distinguish local persistence from reinvasion, identify transmission pathways, or detect emerging lineages. Genomic analysis of *N. perurans* has been hindered by difficulties in axenic culture and contamination from associated microbial communities. We therefore developed a targeted AmpSeq panel for simultaneous genotyping of both *N. perurans* and its kinetoplastid endosymbiont, *Perkinsela*, directly from biological material.

Methods

An AmpSeq panel targeting nuclear and mitochondrial loci from both partners was designed from a draft *N. perurans* genome assembly. This panel was applied to 58 isolates collected from 12 Atlantic salmon aquaculture sites in Scotland and Norway. Sequence reads were processed to generate high-confidence SNP datasets for population genomic analysis. Population structure, clonal diversity, linkage disequilibrium, isolation by distance, and host-symbiont evolutionary congruence were assessed using standard multivariate and phylogenetic approaches.

Results and Discussion

The panel resolved 50 host and 44 symbiont high-confidence SNPs with mean coverage exceeding 150x. The *N. perurans* host population showed weak geographic structuring ($F_{st} = 0.008$; $p = 0.123$) and comparatively high genotype richness (47 MLGs), consistent with substantial regional connectivity. In contrast, the *Perkinsela* showed lower diversity (21 MLGs), stronger clonality and significant geographic structure ($F_{st} = 0.035$; $p < 0.001$). Significant isolation-by-distance was detected in both partners, indicating that dispersal is extensive but not fully homogenised.

Global cophylogenetic analyses supported predominantly vertical transmission between host and symbiont. However, frequent genotype mismatches were observed with 10 of 11 host MLG genotypes represented by multiple isolates harbouring different *Perkinsela* lineages. This indicates that strict co-inheritance is not absolute and that reassortment or lineage switching may occur.

These findings demonstrate that AmpSeq can be a scalable tool to provide actionable genomic surveillance data for Amoebic Gill Disease by differentiating persistence from reinfection, identifying transmission connectivity between farms or regions, and detecting emerging host or symbiont lineages relevant to management and biosecurity.

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CAN LOCAL PROTEIN HYDROLYSATES STRENGTHEN STRESS RESILIENCE IN EUROPEAN SEABASS *Dicentrarchus labrax*?

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Introduction

In intensive aquaculture systems, routine practices including handling, air exposure, transport, and overcrowding impose recurrent physiological challenges that elicit stress responses in fish. These acute challenges can compromise fish health and welfare, predisposing individuals to disease and ultimately result in mortality and economic losses. Concurrently, the reduction of fishmeal (FM) in aquafeeds, driven by sustainability pressures, is altering the dietary supply of essential nutrients, particularly for carnivorous species (e.g., European seabass) and potentially limiting their metabolic capacity to cope with stressors. Animal by-products protein hydrolysates (PH) are emerging as protein-rich ingredient with functional properties, largely attributed to bioactive peptides with antioxidant activity and metabolic regulatory potential. This study aimed to investigate the potential of dietary PH inclusion to antioxidant capacity and metabolic responses in European seabass subjected to an acute stress challenge.

Materials and Methods

Juvenile European seabass were reared in triplicates tanks and fed for 89 days with four plant-based diets: CTRL diet (12.5% fishmeal, FM) and three other diets (SHARK, FISH and SWINE), each including 3% PH derived from blue shark (*Prionace glauca*) skin, fish by-products and swine processed animal protein, respectively, at the expense of FM. At the end of the trial, five fish per tank were immediately sampled, while another five fish were subjected to an acute stress protocol consisting of overcrowding (100 kg m⁻³ for 5 min) followed by air exposure (1 min), and subsequently sampled 1 h post-stress. Plasma and liver were collected to evaluate metabolic and oxidative stress responses.

Results

The diets equally promoted a normal fish growth and ensured high feed efficiency (Rodrigues-dos-Santos et al., 2025). The stress challenge elicited a clear systemic response, characterized by increased plasma cortisol, glucose and lactate levels, along with a reduction in plasma protein. Diet-specific effects were also observed, with the SWINE diet increasing plasma cholesterol levels, whereas the FISH diet resulted in higher plasma lactate concentrations, independently of the stress condition.

Stress also significantly modulated hepatic metabolic activity leading to an increase in the activities of pyruvate kinase (PK) and malic enzyme (ME) under stress conditions, independently of dietary treatment. Fish fed the SWINE diet exhibited even higher PK and ME activities compared to the CTRL group, despite the stress conditions.

In parallel with systemic changes, stress promoted a marked shift in hepatic energy metabolism. In stressed fish, hepatic glucose levels increased in the CTRL group but decreased in the SHARK group, compared to baseline. Moreover, the stress challenge significantly reduced hepatic glycogen and increased hepatic lactate levels only in the CTRL and FISH groups.

Regarding antioxidant responses, under basal conditions, GST activity was higher in fish fed the SWINE diet compared to the other groups, whereas no differences were detected among treatments under stress conditions. In contrast, under basal conditions, CAT activity did not differ among dietary groups, but was significantly increased in the SHARK group under stress. These metabolic and antioxidant responses were reflected in markers of oxidative damage. Lipid peroxidation (LPO) was reduced in fish fed the SWINE diet irrespective of stress, and was also decreased in the SHARK group under stress conditions. In turn, under stress a decrease in protein carbonyl content was only detected in the SWINE group.

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Conclusion

Dietary protein hydrolysates modulated the metabolic and oxidative stress responses of European seabass to acute stress. SHARK and SWINE diets promoted improved metabolic stability, as reflected by better maintenance of hepatic energy reserves and reduced reliance on anaerobic metabolism compared to CTRL and FISH. These diets also enhanced antioxidant status, as indicated by LPO, with SWINE showing additional protection against protein oxidation. Overall, the results suggest that SHARK and SWINE hydrolysates can improve fish stress resilience through coordinated regulation of energy metabolism and oxidative balance, while contributing to reduced dietary FM reliance in plant-based diets for European seabass.

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REPLACEMENT OF FISH PROTEIN HYDROLYSATE WITH SWINE HYDROLYSATE MODULATES MUSCLE PROTEOME AND ALLERGENIC PROTEINS IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

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Introduction

The use of circular protein ingredients in aquafeeds is increasing as aquaculture seeks to reduce dependence on marine resources. Swine protein hydrolysate (SH), produced from swine by-products, has shown potential as a sustainable alternative to fish protein hydrolysate (FH – CPSP90) [1]. However, little is known about its effects on fish muscle proteome and allergenic protein profiles. This study evaluated the impact of replacing FH with SH on the muscle proteome of European seabass (*Dicentrarchus labrax*), with particular emphasis on proteins belonging to allergenic families.

Material and methods

European seabass (IBW=10.7g) were distributed across 12 tanks and fed four experimental diets in triplicate for 96 days: an animal protein-based control diet (Ctrl Pap), a plant protein-based control diet (Ctrl Veg), and two corresponding diets where 3% SH was added at the expense of FH (Pap-3 and Veg-3). Muscle samples (n = 6 per treatment) were analysed using LC-MS/MS proteomics. Differentially abundant proteins were identified using a moderated t-test (p < 0.05; FC 1.5 threshold) [2].

Results and Discussion

In this study, all diets were equally accepted by European seabass (FBW= 47.7 – 50.2g). Dietary SH induced clear proteomic changes, particularly in fish fed the animal protein-based diet. A total of 70 differentially abundant proteins were identified in the Ctrl Pap vs Pap-3 comparison, whereas only 21 proteins differed between Ctrl Veg and Veg-3. Functional annotation revealed alterations in metabolic, regulatory and muscle-structural pathways. Targeted analysis of allergenic protein families showed significant modulation of collagens, myosins, tropomyosins and metabolic enzymes. Notably, parvalbumin, a major fish allergen, was significantly down-regulated (pvalb6; log₂FC = -0.87) in Pap-3 fish when compared to Ctrl Pap fish. In contrast, no reduction in parvalbumin abundance was observed in fish fed vegetal protein-based diets. These findings indicate that the effects of SH depend on dietary background and suggest that the source of dietary protein (animal vs vegetal sources) plays a significant role in shaping proteomic responses to SH inclusion [2].

Conclusions

Replacement of FH with SH modulated the muscle proteome of European seabass and altered the abundance of proteins associated with allergenic families. The reduction of parvalbumin observed in Ctrl Pap vs Pap-3 comparison highlights the potential of dietary strategies to influence allergen-related proteins while supporting the use of circular feed ingredients in aquaculture. Further studies are required to determine whether these proteomic changes translate into reduced allergenicity.

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TIME-DEPENDENT EFFECTS OF ENVIRONMENTAL ENRICHMENT ON JUVENILE EUROPEAN SEABASS: NEURONAL ACTIVITY AND FEEDING BEHAVIOR

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Introduction

European seabass is one of the most commercially important species in the Mediterranean aquaculture sector. Fish reared in captivity are exposed to various factors that may compromise their welfare, such as chronic stressors associated with routine operations and environmental conditions (Rey Planellas et al., 2026). Environmental enrichment (EE) represents a well-established strategy to promote animal welfare by providing novel stimuli that meet behavioral, physiological, and psychological needs (Arechavala-Lopez et al., 2022; Rey Planellas et al., 2026) physiological, morphological and psychological needs, whilst reducing stress and frequency of abnormal behaviours. In fish farms, rearing environments are usually designed from a human perspective and based on economic requirements, mainly for practical reasons for the farmer, with little consideration for animal welfare. Throughout aquaculture production cycles, many farming operations can be stressful for fish, and EE may not only help them cope with these stressful events but also improve their overall welfare. In recent years, increasing interest on the effects of EE in captive fish has focussed mainly on structural enrichment. However, there are many other enrichment strategies that merit attention (e.g. sensorial, occupational, social and dietary enrichment. In aquatic species, structural environmental enrichment, understood as the introduction of physical complexity through objects or environmental modifications, has been shown to enhance cognitive abilities, brain plasticity, and nervous system function. It can also modulate the stress response, reduce stereotypic behaviors, and, in some cases, improve growth and survival (Arechavala-Lopez et al., 2022; Brunet et al., 2022) physiological, morphological and psychological needs, whilst reducing stress and frequency of abnormal behaviours. In fish farms, rearing environments are usually designed from a human perspective and based on economic requirements, mainly for practical reasons for the farmer, with little consideration for animal welfare. Throughout aquaculture production cycles, many farming operations can be stressful for fish, and EE may not only help them cope with these stressful events but also improve their overall welfare. In recent years, increasing interest on the effects of EE in captive fish has focussed mainly on structural enrichment. However, there are many other enrichment strategies that merit attention (e.g. sensorial, occupational, social and dietary enrichment. The effects of environmental enrichment vary depending on the species and its life stage. Despite growing interest in this strategy, there remains a significant lack of knowledge for some of the most relevant species in the industry, such as European seabass (*Dicentrarchus labrax* L.). In this context, our objective is to evaluate the effects of environmental stimulation through physical complexity on the overall welfare of seabass, considering its different components: functional, natural, and emotional welfare.

Materials and Methods

The study was conducted at the experimental facilities located in Port d'Andratx (Balearic Islands, Spain), belonging to the Laboratory of Marine Research and Aquaculture (LIMIA-IRFAP). A total of 4,000 juvenile European seabass (*Dicentrarchus labrax*) with an average body weight of approximately 30 g were randomly distributed among eight experimental marine cages. Four of these cages were structurally enriched, while the remaining four were maintained without enrichment and served as controls. 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). Juvenile individuals were maintained under these experimental conditions for approximately nine months (11/03/2024–18/12/2024; 282 days). Throughout the experiment, four sampling events were conducted at three-month intervals (t0, t3, t6, and t9). At each sampling point, the same parameters were systematically recorded, including biometric measurements (total length and body weight), individual photographs for morphometric analysis (based on distances between 24 anatomically relevant landmarks), and telencephalon and muscle tissue samples. These samples were subsequently used to assess oxidative status through the activity of antioxidant enzymes, as well as to quantify the expression of genes of interest related to stress,

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neuronal activation, neurogenesis, and neuroplasticity. In addition, feeding response was recorded daily for each cage using a consumption index throughout the entire experimental period. Finally, six months after the start of the experiment, individuals were implanted with acoustic transmitters, allowing their activity to be monitored via acoustic telemetry within the rearing cages.

Results and discussion

The results showed no significant differences between treatments in the biometric or morphometric parameters evaluated, indicating that structural environmental enrichment (EE) does not affect growth, body condition, or morphology in juvenile seabass. Likewise, no effects of enrichment were detected on oxidative stress markers in either muscle or telencephalon, suggesting the absence of a physiological stress response associated with the experimental conditions. In contrast, gene expression analysis in the telencephalon generally revealed higher expression levels in enriched individuals for genes associated with neurogenesis, neuronal activation, and synaptic plasticity. Moreover, a time-dependent expression pattern was observed exclusively in enriched individuals, characterised by a progressive increase in gene expression that peaked during the intermediate phase of the experiment (t3–t6), followed by a decline towards the end of the experimental period (t9). This pattern suggests that structural EE induces an initial activation of neural processes related to memory, neuronal activity, and neuroplasticity, which subsequently decreases after prolonged exposure, likely as a consequence of habituation to a stable and unchanging enriched environment. In addition, structural EE had a positive effect on feeding response, which was higher than in the control group during the last three months of the experiment in enriched individuals. Overall, these results indicate that structural EE transiently modulates neural activity without compromising the general physiological status of individuals, evidencing a temporal window of response to environmental stimulation under static conditions. This suggests that stimulus renewal may be necessary to sustain this positive response over time.

Acknowledgment

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FROM INVISIBILITY TO NECESSITY: IMPROVING PUBLIC PERCEPTION OF AQUACULTURE IN SPAIN THROUGH A LONG-TERM COMMUNICATION STRATEGY

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Introduction

Despite its strategic role in food production, aquaculture has traditionally suffered from limited public awareness and social acceptance in Spain. In 2019, research commissioned by APROMAR revealed that most citizens had little understanding of aquaculture and often associated fish farming with negative concepts such as artificial production, overcrowding, lower product quality and environmental concerns. These perceptions represented a barrier to social licence and sector development.

To address this challenge, APROMAR launched the Strategic Communication Plan for Spanish Aquaculture (PLECA), aiming to increase public awareness, improve perception of aquaculture products and strengthen social support for the sector.

Methods

The communication strategy was implemented between 2020 and 2025 and combined three complementary approaches:

1. Public education on what aquaculture is, why it is necessary and how it is practiced in Spain.
2. Development of science-based communication materials, including digital content, media outreach, expert collaborations and search-engine positioning.
3. Promotion of sector initiatives reinforcing transparency and credibility, including sustainability reports and fish welfare guidelines.

The strategy also introduced a communication shift from the widely recognised term “fish farming” (piscifactoría), strongly associated with negative perceptions, towards the broader concept of “aquaculture”, accompanied by messages focused on sustainability, animal welfare, food security and scientific evidence.

Public perception was monitored through periodic national surveys conducted between 2019 and 2025.

Results and Discussion

The results indicate a substantial improvement in public awareness and acceptance of aquaculture.

Spontaneous awareness of aquaculture increased from 1.9% in 2019 to 11.4% in 2025. Total awareness reached 51.8%, while aided recognition reached 77.4%.

Among citizens familiar with aquaculture, 75.7% reported a positive or very positive opinion of the activity, and 97.7% considered aquaculture necessary or very necessary for society.

Perceptions of Spanish aquaculture were particularly favourable. Seventy-eight percent of respondents considered Spanish aquaculture better than aquaculture in less developed countries, while 94.3% considered it equal to or better than aquaculture in other developed countries.

The findings suggest that long-term communication strategies based on transparency, scientific evidence and sector commitment can contribute significantly to strengthening social licence. Results also indicate that communication effects are cumulative, with the most significant improvements occurring during the final stages of the programme.

Conclusions

The Spanish experience demonstrates that improving public perception of aquaculture requires more than isolated campaigns. Sustained communication efforts, supported by credible sector actions and measurable evidence, can transform aquaculture from a poorly understood activity into one perceived as necessary, responsible and socially valuable.

This case study may provide useful lessons for other aquaculture-producing countries seeking to strengthen public trust and social acceptance.

TAILORING THE NUTRITIONAL QUALITY OF *Pavlova gyrams* THROUGH DYNAMIC LIGHT MANAGEMENT FOR MARINE AQUACULTURE

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Introduction

Microalgae are essential in marine hatcheries as a primary feed source for fish, bivalve and crustacean larvae. Improving the nutritional quality of microalgal biomass is becoming increasingly important to meet the specific nutritional requirements of aquaculture species. *Pavlova gyrams* is recognised as a valuable microalga for marine aquaculture because of its balanced lipid profile and bioactive pigments (Almeyda et al. 2023). Light is one of the main environmental factors regulating biomass composition, yet its potential as a cultivation tool to tailor the nutritional profile of marine microalgae remains poorly understood. This research evaluated the effect of the light-shift from 125 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to moderate and high light on biomass, lipid and fucoxanthin productivity in *P. gyrams*.

Materials and methods

P. gyrams was purchased from the Basque Microalgae Culture Collection (BMCC), Spain. Two independent batch experiments were conducted in 25 L tubular photobioreactors (T-PBRs) each lasting 13 days. Cultures were initially maintained at 125 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and, on day 8, light intensity was increased either to 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. This modification aimed to prevent self-shading associated with biomass accumulation, thereby maintaining a relatively uniform light availability per cell. Biomass, productivity, total lipid content, fatty acid composition and fucoxanthin productivity were monitored to assess changes in biomass quality under different light regimes.

Results

The maximum biomass concentrations observed were 5.32 ± 0.13 and $6.52 \pm 0.07 \text{ g} \cdot \text{L}^{-1}$, four days after shifting the light intensity to 300 and 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. However, maximum biomass productivities were recorded primarily prior to this shift, corresponding to the exponential growth phase of the cultures. Overall, a moderate increase in light intensity to 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ resulted in unchanged fatty acid productivity, a stable triacylglycerol-to-phospholipid ratio, and steady productivities of EPA, DHA, and fucoxanthin. Conversely, although a further increase to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ sustained growth acceleration, it increased the triacylglycerol (TAG) fraction and reduced the productivities of both fucoxanthin and EPA.

Discussion

DHA and EPA were predominantly associated with the polar lipid (PL) fraction, that is especially relevant for aquaculture, as these membrane – associated fatty acids are more efficiently utilized by fish larvae and are therefore preferred in hatchery systems (Guedes et al. 2010) which resorts more and more often to microalgae as feed; the lipid composition of such microalgae is a critical factor with regard to the fish growth rate upon ingestion. The aim of this work was thus to study the influence of light intensity on the lipid profile of a known microalga, *Pavlova lutheri*. Several semi-continuous cultures were carried out, and biochemical parameters such as lipid, protein, carbohydrate, and chlorophyll contents were quantified. Lipids were specifically fractionated into classes by TLC, and those in each class were subjected to GC afterwards in an attempt to ascertain their fatty acid profile. Evidence was consequently provided which showed that cultures grown under low light intensity (9 W m⁻²). However, an excessive photon flux increased the TAG fraction in the desirable fatty acids (DHA and EPA). Consistent with previous studies, the highest light intensity reduced fucoxanthin productivity, whereas moderate light maintained pigment production (Kurpan Nogueira et al. 2015; Gao et al. 2020). *Nannochloropsis oceanica* and *Phaeodactylum tricornutum* was compared to study their suitability for biotechnological applications. Photoautotrophic batch cultures grown at 20 °C and 50 mmol photons m⁻² s⁻¹ showed that *N. oceanica* had the least TAG content and TAG productivity of the three microalgae. Hence, effects of temperature and light intensity on growth rate and accumulation of TAG were subsequently assessed only in *I. galbana* and *P. tricornutum* by cultivation at 20 and 30 °C under 50, 300 and 600 mmol photons m⁻² s⁻¹. Although *P. tricornutum* did not grow at temperatures higher than 20 °C, an increase in both TAG content (from 28.37 to 39.53%).

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These findings demonstrate that dynamic light management can modulate carbon partitioning (Chadova 2024) while sustaining the production of high-value compounds, highlighting its potential to optimize *P. gyrans* cultivation for marine aquaculture and other biotechnological applications.

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LEGISLATION OF AQUACULTURE STATUS AND PERSPECTIVES IN ITALY

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Introduction

Since the early 2010s, the European Union has identified aquaculture as a strategic component of sustainable Blue Growth. The Common Fisheries Policy¹ and the EU aquaculture guidelines² promote a sector that is economically competitive, environmentally sustainable and capable of supporting employment and food security in coastal and rural communities. The 2020 Farm to Fork Strategy further recognises farmed seafood as a potentially low-carbon source of protein within a more sustainable European food system³. However, aquaculture remains marginal within the Italian economy, representing approximately 2.45% of agricultural GDP and showing a decreasing trend. Moreover, recent research estimates that around 80% of Italian aquaculture companies are experiencing financial stress, which limits their ability to invest in innovation, environmental improvements and new production technologies⁴. This contrast raises a central question: Why has Italian aquaculture not achieved the growth, competitiveness and sustainability envisioned by European policy?

Aims

This study aims to investigate the factors limiting the sustainable development of Italian aquaculture, with particular attention to the economic and financial vulnerability of aquaculture farms, the complexity and fragmentation of the regulatory framework, the interaction between European and Italian and regional legislation and the cost of complying with environmental and water-quality requirements. The analysis also considers whether greater policy coordination, targeted financial support and incentives for technological innovation could facilitate the transition towards a more resilient and sustainable Italian aquaculture sector.

Results

Currently the analysis identifies a gap between European strategic ambitions and the operating conditions faced by Italian aquaculture businesses. Financial stress reduces farms' ability to invest in technologies that could improve productivity, water quality, energy efficiency, biosecurity and waste management. Small and medium-sized farms are particularly exposed because regulatory compliance and technological investments frequently involve high initial costs. Italian aquaculture operates within multiple and partly overlapping European and national rules. At EU level, the Common Fisheries Policy seeks to ensure the long-term environmental, economic and social sustainability of fisheries and aquaculture. However, aquaculture businesses must also comply with legislation concerning water quality, environmental protection, animal health, food safety, spatial planning and the use of coastal or inland waters. At the national level, fisheries and aquaculture are governed through several legislative measures, including Legislative Decree 4/2012, reorganising national fisheries and aquaculture legislation⁵; Act 164/1998, addressing fisheries and aquaculture activities and financial measures⁶; Legislative Decree 154 of 26 May 2004, promoting the sustainable modernisation of the sector⁷. The coexistence of European, national, regional and local responsibilities may result in complex authorisation procedures, administrative burdens, regulatory uncertainty and delays in business investment. Aquaculture development depends on the quality and availability of water resources. However, agricultural, environmental and aquaculture policies do not always address cumulative pressures on water bodies in an integrated way. Closer alignment between the Common Agricultural Policy and the Water Framework Directive⁸ could improve the management of nutrient loads originating from both agriculture and aquaculture. The Water Framework Directive could consequently function not only as a compliance mechanism, but also as an operational framework for identifying where and how sustainable aquaculture production can expand. Environmental regulation does not necessarily prevent aquaculture growth. If combined with appropriate support, it can encourage nutrient recovery and recycling; recirculating aquaculture systems; integrated multi-trophic aquaculture; improved waste and water management and renewable-energy applications. The EU's more recent aquaculture guidelines recognise administrative burden, access to space and water, environmental performance, climate adaptation, innovation and access to knowledge as interconnected priorities. They call for a sector that is more resilient, competitive and environmentally sustainable. Locally produced seafood may reduce dependence on imported products, especially fresh fish transported over long distances by air. It can also strengthen short supply chains, improve food security and create employment in coastal and rural communities. The Common Fisheries Policy explicitly links aquaculture with food availability, employment, economic development and reduced dependence on seafood imports. EU policy also recognises that sustainable farmed seafood can contribute to a lower-carbon food system⁹.

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Conclusion

The limited growth of Italian aquaculture cannot be attributed to a single factor. It reflects the interaction of financial fragility, administrative complexity, regulatory fragmentation, environmental obligations and insufficient investment capacity. For further steps in the alignment of legislation on aquaculture, a view encompassing all related aspects in the diverse aquaculture sector is needed. European and Italian policies formally support sustainable aquaculture, but many businesses lack the financial and institutional capacity required to translate these objectives into practical innovation. Consequently, environmental compliance may be perceived as an additional cost rather than an opportunity to improve efficiency and competitiveness. The legal framework should be predictable also at the level of permitting authorities and not influenced by the political situation. Unlocking the potential of Italian aquaculture requires simplified and more predictable authorization procedures; greater coordination between European, national and regional policies; closer integration of agricultural, water and aquaculture management; targeted financial assistance for financially vulnerable farms. The sustainable transition of Italian aquaculture will depend not only on stronger environmental rules, but also on ensuring that farms possess the financial, technological and institutional capacity to implement them.

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PHYSIOLOGICAL STRESS RESPONSES OF *Penaeus vannamei* UNDER DIFFERENT LIGHT CONDITIONS

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Introduction

Studies have shown that light intensity and color can induce stress in the shrimp *Penaeus vannamei*, as evidenced by behavioral responses such as tail flips, looping movements and thigmotaxis. The findings suggest that orange light wavelength (600nm) is preferred by the shrimp and does not trigger stress compared to other light conditions (Pichon et al., 2025). However, these results are primarily behavioral. This raises the question of how such light conditions affect the shrimp from a physiological perspective. In particular, this can be investigated using physiological stress markers such as respirometry and blood glucose levels.

Material and Methods

For each respirometry trial, we test groups of four shrimp. Each shrimp is placed in its own metabolic chamber, where oxygen levels are continuously recorded by a data acquisition system. The shrimp are successively exposed to five different light colors (white, orange, blue, green, yellow) for a defined period of time. Each experiment is repeated five times. Light treatments order has been randomized to minimize potential bias. Before starting respirometry trial, shrimp are acclimated during 5 days under darkness within a recirculating aquaculture system (RAS).

Between successive respirometry runs, aeration is restored for 5 minutes to reoxygenate the system, during which the light is turned off. At the end of this period, the aeration is turned off for 15 minutes, with the next light condition initiated simultaneously. Except during these successive illuminated periods of 15 minutes, the experiment is conducted in darkness.

For the experiment measuring hemolymph glucose levels, shrimp are maintained in a separate recirculating aquaculture system (RAS). Each tank, containing 15 shrimp, is exposed to a specific light condition for one day. At each time point (T+15 min, T+30 min, T+2 h, T+4 h, and T+6 h), three shrimp are sampled for hemolymph collection and subsequent glucose analysis. The glucose results obtained will be compared to those obtained from Apriyadi et al., 2023.

Results and Discussion

Based on behavioral results reported by Pichon et al., 2025, an increase in oxygen consumption is expected under white, yellow, and green light conditions, whereas a decrease is anticipated under orange light conditions. Similarly, regarding hemolymph glucose levels, findings from Apriyadi et al., 2023, suggest higher glucose concentrations under white, yellow, and green light, with a reduction under orange light conditions. However, it will be particularly informative to examine how these responses evolve over time, especially in relation to potential habituation effects.

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INFLUENCE OF SHELTER AND RESTRICTED FOOD ON PHYSIOLOGICAL STRESS AND PREDATION IN *Penaeus vannamei*

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Introduction

This study aims to assess the influence of shelter placement and undernutrition on physiological stress and predation in *Penaeus vannamei*. In this species of major aquaculture interest, environmental and nutritional conditions strongly modulate the behavior, physiology and resistance to external aggressions. The main hypothesis is that the presence of shelter could improve the welfare and reduce the risk of predation, while undernutrition is likely to increase vulnerability by weakening energy reserves and shrimp ability to cope with stress. So, a higher oxygen consumption, a peak in blood sugar and a lower survival rate in shrimp with undernourished and homeless conditions compared to shrimp with more shelter and feeding would be studied.

Material and Methods

24 tanks were prepared in the same recirculated water system. 4 treatments were randomly applied (ie 6 replications per treatment) to the different tanks for 21 days (Table 1).

Shrimp were fed twice daily in accordance with their feeding condition. Water physico-chemical parameters (pH, Nitrite, Salinity, Temperature) were monitored twice a week throughout the experiment.

The physiological stress parameters measured were:

- Oxygen consumption,
- Hemolymph glucose level,
- Survival rate.

Results and Discussion

The first average results observed are presented in Table 2.

Lower oxygen consumption and a best survival rate in shrimp with shelter compared to homeless shrimps may show that the presence of refuges reduces stress and predation in *P. vannamei*.

A higher glucose concentration in the hemolymph, along with an increased specific growth rate in overfed shrimp, may indicate that while overfeeding promotes growth, it could be detrimental to their overall health.

These results provide a better understanding of how habitat structure and nutritional status interact to determine juvenile survival.

In perspective, this work could provide useful recommendations for the breeding and growth of *P. vannamei*, by combining enrichment of the environment and food management to improve welfare, robustness and survival.

Table 1: Summary of experimental conditions

Shelter	Overnutrition (9% of the tank biomass)	Undernutrition (4.5% of the tank biomass)
Presence	10 shrimp of 0.8g minimum	10 shrimp of 1g minimum
Lack	10 shrimp of 0.8g minimum	10 shrimp of 1g minimum

Table 2 : First average results

Feeding condition	Shelter	Oxygen consumption (mgO ₂ g ⁻¹ h ⁻¹)	Hemolymph glucose level (mg dL ⁻¹)	Survival rate (%)	Specific growth rate (% day ⁻¹)
Overnutrition	Lack	0.80 ± 0.13	59.58 ± 19.69	56.67 ± 10.33	3.27 ± 0.71
Overnutrition	Presence	0.82 ± 0.15	55.17 ± 20.95	58.33 ± 11.69	3.47 ± 0.65
Undernutrition	Lack	0.95 ± 0.18	32.00 ± 8.51	60.00 ± 16.73	1.51 ± 0.86
Undernutrition	Presence	0.87 ± 0.14	33.57 ± 11.01	70.00 ± 8.94	2.15 ± 0.48

PERFORMANCE OF MARINE ANAMMOX UNDER RAS CONDITIONS: EFFECTS OF OXYGEN AND NITROGEN

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Introduction

As aquaculture production intensifies, concerns regarding nutrient discharges to the environment are increasing. Recirculating aquaculture systems (RAS) enable high water reuse while maintaining controlled farming environments (Van Rijn 2013). In RAS aerobic biofilters, nitrifying bacteria oxidize the ammonium (NH_4^+) excreted by the fish into nitrate (NO_3^-) via nitrite (NO_2^-). NO_3^- is then typically removed through unsustainable water exchanges or converted to nitrogen gas (N_2) via complex denitrification (Chen 2002, Preena et al. 2021).

The anammox (anaerobic ammonia oxidation) process has emerged as a cost-effective and environment-friendly nitrogen removal alternative, where NH_4^+ and NO_2^- are directly converted into N_2 (Kartal et al. 2012). We previously demonstrated that the marine anammox bacteria *Candidatus* Scalindua effectively removed NH_4^+ and NO_2^- from synthetic RAS wastewater under controlled laboratory conditions (Micolucci et al. 2023).

Material and methods

To bridge the gap between controlled laboratory conditions and real-world applications, we further evaluated the performance of *Ca. Scalindua* under RAS-relevant conditions, characterized by low levels of NH_4^+ and NO_2^- ($<1 \text{ mg.L}^{-1}$), elevated NO_3^- (up to $400\text{--}1000 \text{ mg.L}^{-1}$) and the presence of oxygen (O_2), necessary for the fish and the nitrifying bacteria. Upflow reactors were operated under these RAS conditions and monitored over time. Nitrogen removal performance was assessed from influent and effluent concentrations, and microbial community dynamics were analysed using 16S rRNA gene sequencing and FISH.

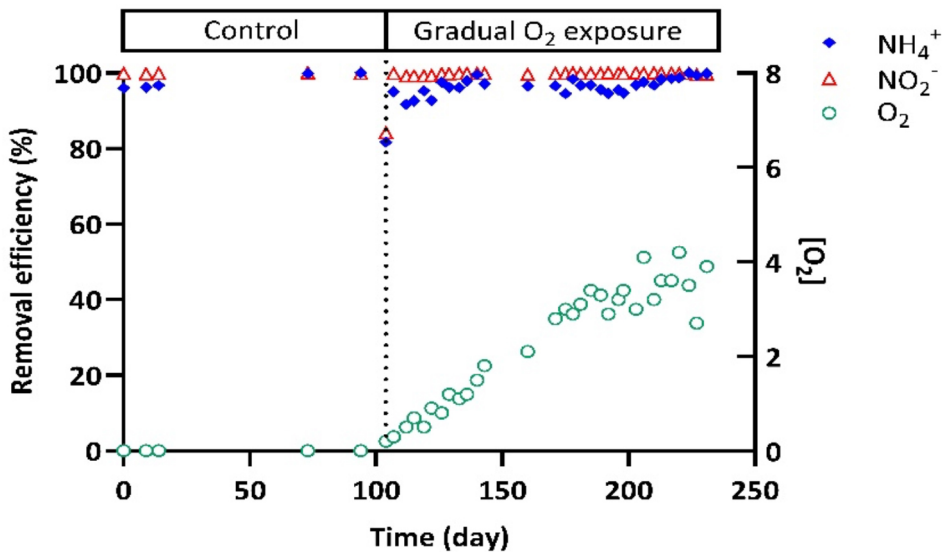


Figure 1. Performance of the marine anammox *Ca. Scalindua* under increasing O_2 concentrations.

(Continued on next page)

Results and discussion

High nitrogen removal efficiencies (>70%) were maintained at low substrate concentrations typical of RAS by increasing flow rates to sustain nitrogen loading and maintaining trace elements supplementation (preliminary results). Elevated NO_3^- concentrations encountered (up to 1000 mg.L^{-1}) did not affect nitrogen removal but reduced the relative abundance of *Ca. Scalindua* in the granule (Roques et al. 2024, Roques et al. 2026). Furthermore, nitrogen removal efficiencies remained high and stable when the biomass was gradually exposed to increasing O_2 concentrations up to 4.5 ppm, the highest level tested so far (Figure 1). These results suggest the potential applicability of anammox under real RAS conditions in marine environments, despite its characterization as a strictly anaerobic process, suggesting a higher tolerance to oxygen under RAS conditions. Further validation at pilot scale is required to confirm long-term stability and process integration.

Acknowledgments

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MODES OF ACTION OF POSTBIOTICS IN AQUACULTURE FROM PATHOGEN INHIBITION TO IMMUNE AND INTESTINAL BARRIER FUNCTION

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Introduction

Postbiotics are preparations of inanimate microorganisms and/or their components that confer a health benefit on the host. They include microbial structures and metabolites such as organic acids, peptides and bacteriocins, all contributing to biological functionality. In aquaculture, the need for feed-additives that are stable during production, safe to handle and effective across species is increasing. At the same time, cultured aquatic animals are exposed to multifactorial challenges, including pathogens, environmental stress and gut health disturbances. This requires solutions acting via multiple biological pathways. This study aimed to characterise the modes of action of a postbiotic using complementary in vitro, ex vivo and in vivo approaches.

Materials and methods

In vitro antibacterial activity was assessed using co-cultivation assays with industry burdening aquatic pathogens including *Vibrio*, *Aeromonas*, *Yersinia* and *Streptococcus* species. Cell-free supernatant (CSF) was used to isolate the effects of soluble components. An ex vivo intestinal explant model was applied to the final product and the CFS to evaluate immune and barrier responses through cytokine expression (IL-1 β , IL-8, TNF α , IL-10) and tight junction markers (occludin, claudins) and to confirm previous in vitro observations of the same readouts in other cellular models. In vivo trials across aquaculture species evaluated robustness under pathogenic and environmental challenges.

Results and discussion

The postbiotic additive showed broad antimicrobial activity, indicating direct suppression of pathogens via soluble bioactive compounds. Ex vivo results demonstrated activation of innate immune pathways with concurrent upregulation of IL-10, suggesting balanced immune modulation with anti-inflammatory control. Increased expression of tight junction markers indicated improved epithelial integrity, consistent with enhanced barrier function and reduced permeability. In vivo trials confirmed improved resilience under challenge conditions, reflecting the integration of antimicrobial, immune and barrier effects. These complementary mechanisms are relevant across aquaculture systems, where animals face overlapping stressors.

Conclusion

Postbiotics act through interconnected mechanisms including pathogen inhibition, modulation of inflammatory responses and reinforcement of intestinal barrier function. Their stability and compatibility with feed production make them promising functional additives for aquaculture. The consistency of responses across experimental models indicates that their benefits are not species-specific but represent general physiological pathways. Postbiotics therefore offer a versatile and scalable solution to support health and performance across aquaculture species and production systems.

PHYSIOLOGICAL RESPONSE OF *Salmo carpio* L. BROODSTOCK FOLLOWING MANUAL STRIPPING

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Introduction

The Carpione (*Salmo carpio* L.) is an endangered salmonid endemic to Lake Garda and represents a promising candidate for aquaculture diversification in the Autonomous Province of Trento, Italy. Carpione inhabits deep pelagic waters at depths exceeding 150 m. Its reproductive cycle involves seasonal migrations from the central-southern basin toward northern spawning grounds, where it seeks gravelly-sandy substrates at depths of 150 – 200 m, typically 200 – 500 m offshore (Raunich et al., 1990). Sexual maturity is generally reached at approximately 3 years for males and 4 years for females (Melotto & Alessio, 1990). While spawning phenology remains a subject of debate, two distinct periods are generally recognized: a primary winter peak (December–February) and a secondary summer event (July–August) (Gandolfi et al., 2006). Interestingly, some females may spawn twice within a single year, a phenomenon likely driven by rapid post-spawning vitellogenesis (Melotto & Alessio, 1990). Following reproduction, adults migrate back to the southern basin to feed (Raunich et al., 1990). Despite its potential, limited understanding of the species' reproductive physiology currently constrains both commercial production and conservation efforts.

The aim of the “Filiera Astro” project is to improve the local aquaculture industry through the adoption of precision fish farming principles, a better understanding of farm-environment interactions, and strategies to limit pressure on ecosystems. Additionally, the project focuses on the reuse of fish-farming by-products and the application of new technologies to reduce feed waste and environmental impacts. A part of the project focuses on developing farming protocols for *S. carpio*. This study assessed reproductive performance and key physiological indicators in captive broodstock during the 2025 spawning season, with emphasis on endocrine status, histological analysis, and stress responses associated with stripping procedures.

Material and methods

Reproductive monitoring was conducted from February to July 2025. A total of 479 females and 264 males were spawned, yielding 64 L of eggs with an average fertility of 40.4%. A marked production peak occurred in April. To evaluate physiological responses to handling and reproduction, females were sampled at three time points (15 individuals per group): prestripping (PRE), 24 h poststripping (POST1), and 21 days poststripping (POST21). The following parameters were monitored in fish plasma: 17 β -estradiol (E2), testosterone (T), progesterone (P4), glucose, superoxide dismutase (SOD), catalase (CAT), and glutathione Peroxidase (GPx). Ovaries were analysed through histology using hematoxylin and eosin Y (H&E) and periodic acid-Schiff (PAS) staining procedures to evaluate the ovarian cellular structure.

Results and Discussion

Contrary to published reports of first maturation occurring at age 3 for males and age 4 for females, a substantial proportion of 2-year-old individuals (62%) in this study reproduced successfully in captivity. However, these younger fish exhibited significantly higher post-spawning mortality compared to 3-year-old broodstock. This suggests that precocious maturation—likely accelerated by consistent feeding and reduced energetic costs in a controlled environment—may compromise long-term survival. For commercial production, delaying first maturation until age 3 may enhance both animal welfare and overall reproductive efficiency.

Regarding physiological indicators, plasma concentrations of estradiol, testosterone, and progesterone did not differ significantly between groups, indicating stable steroidogenic activity throughout the sampling period. Histological analysis confirmed ongoing reproductive activity, with ovaries at various maturity stages observed in POST21 females and no evidence of anatomical damage resulting from the stripping procedure.

In contrast, cortisol levels increased significantly ($p < 0.05$) by POST21. This elevation was accompanied by increased activity of antioxidant enzymes, specifically superoxide dismutase (SOD) and catalase (CAT), revealing a delayed stress and oxidative response. Notably, glucose levels remained stable.

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These findings indicate that while the stripping procedure does not acutely suppress steroid production or cause physical tissue damage, the cumulative impact of handling and reproductive effort induces measurable stress after three weeks. Younger broodstock appear particularly vulnerable, highlighting the necessity of optimizing management practices and minimizing handling frequency to preserve the welfare and reproductive performance of *S. carpio*.

Acknowledgment

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***Paecilomyces variotii* SUPPORTS GROWTH AND HEALTH OF ATLANTIC SALMON (*Salmo salar*) POST-SMOLT**

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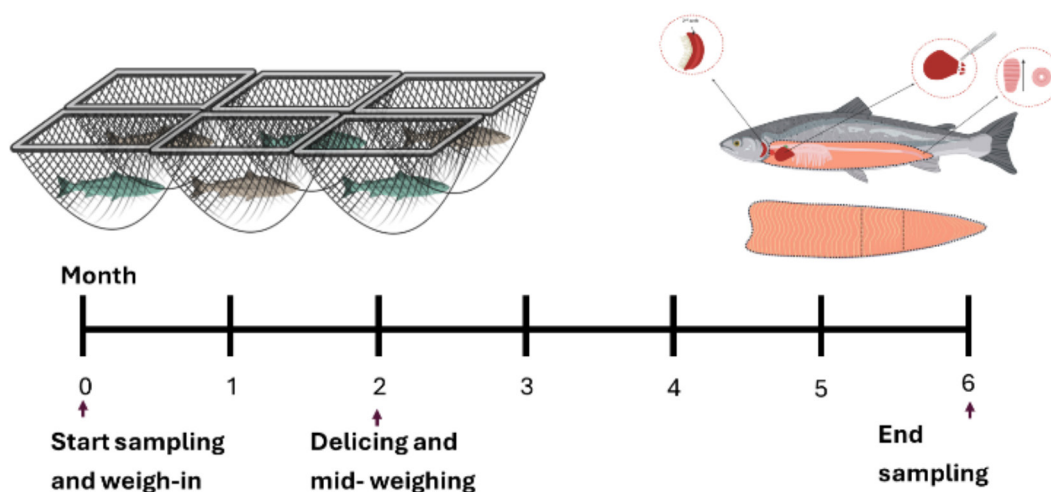
Introduction

The Norwegian aquaculture industry is faced with several challenges, including limited access to high quality feed ingredients, and multi-stressor conditions such as changing environmental conditions and pathogen exposure leading to fish mortalities and economic losses to the industry. Consequently, there is a need for sustainable protein sources for aquafeeds with high nutritional value that also contain bioactive components with health-promoting effects. As part of the solution, microbial ingredients (MIs) such as filamentous fungi are gaining interest since they can convert low-value organic biomass into high-value nutrients, as well as they contain microbe-associated molecular patterns that can modulate the fish immune response. In addition, MIs typically have a high yield, require minimal arable land, have a low carbon footprint, and have a high potential for up-scaling to a commercial level. Thus, the use of MIs aligns with the Norwegian government's goal that all feed ingredients should come from sustainable sources, and that 25% of these should be locally sourced by 2034.

Paecilomyces variotii (PEKILO®) is a filamentous fungus that can be fermented from forestry side-streams and holds promise in novel functional aquafeeds. *P. variotii* has high nutritional value with a protein content of 60-70% (dry matter basis) and a favorable amino acid composition. Additionally, *P. variotii* is rich in β -glucans, mannoproteins and nucleotides, which have been shown to improve short-term growth performance, modulate microbiota composition and promote fish health and welfare (Hadiuzzaman et al., 2022; Hooft et al., 2024; Hossain et al., 2020; Mensah et al., 2024; Mensah et al., 2025). These observations indicate that *P. variotii* can be a promising sustainable protein source for aquaculture-related species, and to improve fish resilience to cope with multi-stressor conditions. Therefore, the aim of this study is to investigate the long-term effects of continuous feeding of feed containing *P. variotii* on growth performance, health and filet quality of post-smolts Atlantic salmon (*Salmo salar*).

Materials and Methods

A total of 720 vaccinated post-smolt Atlantic salmon were fed two diets in triplicates (Figure 1), containing 0% or 10% *P. variotii*. Prior to the start of the distribution to the net pens, the immune related tissues from 10 fish were sampled to establish a baseline. Then, fish were distributed into six net pens (5 x 5 meters, 120 fish per net pen). The fish were fed for six months, and at the end of the experiment, tissues, plasma and weight were taken from four fish per net pen and analysis of filet quality was performed of 10 fish per net pen. Preliminary results show that *P. variotii* supports growth performance. The experimental period ended in January 2026; fish growth performance, product quality, and multi-omics results from head kidney, spleen, and distal intestine will be presented at Aquaculture Europe 2026.



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EVALUATION OF HIGH-LEVEL SUBSTITUTION OF FISH MEAL WITH DEFATTED BLACK SOLDIER FLY (*Hermetia illucens*) LARVAL MEAL IN JUVENILE EUROPEAN SEABASS (*Dicentrarchus labrax*) DIETS: IMPACT ON GROWTH PERFORMANCE, INTESTINAL STRUCTURE, AND GENE EXPRESSION

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Introduction

Fish meal (FM) has long been considered the ideal protein source for carnivorous fish production, due to its well-balanced nutritional profile, high digestibility, and palatability. However, due to its limited global availability and environmental concerns, current research is focused on reducing the reliance on FM in aquafeeds by substituting it with more available, eco-friendly, and cost-efficient protein alternatives. Significant progress has been made in utilizing alternative proteins derived from terrestrial plants. However, traditional plant ingredients used as substitutes often contain several anti-nutritional factors and could present conflicts, as some these resources are intended for human consumption. In recent years, after the European Commission approved the use of specific insect meals as feed materials for aquafeeds. Black soldier fly (*Hermetia illucens*) larvae meal (BSF) has gained significant attention from the scientific community as a sustainable and novel protein source

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, all fish were starved for 48 h to evacuate the digestive tract, anesthetized with phenoxyethanol: ethanol (1:1), and individually weighed to assess growth performance. A total of 19 fish were randomly selected from each tank and pooled. Four fish were euthanized by anesthetic overdose, and samples of skeletal muscle, perivisceral fat, the intestinal tract, and liver were collected to calculate the somatometric indices. Histological samples were preserved in 10% buffered formalin (with sodium phosphate and sodium hydroxide; pH 7.4) until analysis. Additionally, fillet samples were collected from the same fish and stored at -20 °C. Another five fish from each tank were used for whole-body composition analyses. Additionally, 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. 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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Results and Discussion

At the end of the feeding trial, fish growth performance and feed utilization indices were not significantly affected by the FM substitution up to 60%. Additionally, the whole-body proximate composition showed minimal variation among treatments. Significant differences were observed in the fillet fatty acid composition, showing increased n-6 polyunsaturated fatty acids and reduced long-chain n-3 PUFA (EPA and DHA) associated with higher BSF content in the diets. While the histological assessment showed no alterations in intestinal morphology and no signs of liver inflammation. Interestingly, the largest lipid deposits were observed in the Control and BSF 60% groups, while the smallest deposits were noted in the BSF 20% group. The remaining dietary treatments had intermediate values ($p < 0.05$). A multi-tissue partial least-squares discriminant analysis (PLS-DA) of gene expression revealed that at 60% inclusion of defatted black soldier fly (BSF) meal, there was notable immune modulation. This was characterized by increased pro-inflammatory markers and decreased regulatory cytokines in the intestine. 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.

Acknowledgment

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ECOLOGICAL STOICHIOMETRY AND NUTRIENT REDUCTION STRATEGIES FOR SUSTAINABLE BUT INTENSIVE HYBRID POND SYSTEMS – CONCEPTS FOR REGENERATIVE AQUACULTURE

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Introduction

Hybrid pond systems integrate intensive and extensive aquaculture to improve resource efficiency, using the surrounding pond as a natural biofilter for waste assimilation. However, carbon and zooplankton management in this outer pond is largely overlooked despite its central role in nutrient processing. Applying ecological stoichiometry and PEG (plankton ecology group) model principles could enhance biofilter function and sustainability, potentially aligning hybrid ponds with EU regulatory goals and regenerative aquaculture concepts. Nonetheless, current evidence shows that improved water quality is not guaranteed, and strict nutrient management (N, P) remains essential to meet EU Water Framework Directive standards. In this study, we explored ecological concepts to abate the long-term eutrophication risk associated with hybrid ponds, which are gaining popularity as an ecological approach to aquaculture.

Materials and Methods

Using fish-free pond-water mesocosms, we examined the response of algal growth to additions of fish feces and carbonaceous material under realistic or challenging dilution scenarios during both dynamic and steady states (Experiments I-II). Using pond living labs observational study, we examined uncertainties in zooplankton body composition under variable bottom-up and top-down pressures, mediated by nutrient stoichiometry and fish stock index (FSI), respectively (Experiment-III).

In experiment-I, the objective was to assess graded levels of N and P inputs via fish feces at the expense of organic C on the dynamic and steady states of mesocosms containing pond water.

In experiment-II, the objective was to assess the behavior of pond water mesocosms at graded levels of stoichiometric effects from C:P and N:P molar ratios. For this purpose, two isonitrogenous fish feces and two isocarbonaceous feeds were used, with low and high C:P or N:P ratios, respectively.

In experiment-III, using temperate carp ponds across the vegetative season as aquatic living labs, the objective was to explore the relative variability of amino acid and fatty acid contents in zooplankton (additionally, body condition factor) under highly variable water nutrient stoichiometries (molar DIN:TP, DOC:DIN, DOC:TP) and fish stock index (FSI). It was done to understand any hidden sensitivities in zooplankton's nutritional ecology (exerting top-down control on algae) to variable nutrient inputs and predation pressure in its environment.

Results

Experiment I, which examined graded levels of N and P at the expense of cellulose-lignin type organic C, showed P-enrichment strongly explained treatment differences, with elevated C-inputs partially suppressing algal proliferation.

Experiment-II, which examined stoichiometric effects of molar C:P (at fixed N) and N:P (at fixed C) ratios, indicated that solids with lower C:P and N:P ratios markedly accelerated chlorophyll and cyanobacterial pigment accumulation, with N:P exerting the strongest influence on C:P. At high C but low P, brownification or dissolved oxygen risks occurred.

Experiment-III revealed that zooplankton can maintain their body amino acid composition (basal maintenance) under fluctuating bottom-up/top-down pressures, except for specialized tyrosine-rich storage proteins. The fatty acids 20:4n-6, 20:5n-3, and 22:6n-3 in zooplankton exhibit great uncertainties under imbalances in DIN:TP and FSI, essential for propagation— further confirmed by direct measurements of zooplankton body condition factor.

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Group name	Composition*	Solids-to-water Dilution (w/w)	C input (mg l ⁻¹)	N input (mg l ⁻¹)	P input (mg l ⁻¹)
Experiment-I (mesocosm)					
FF	100% fish feces	1: 10000	47.80	3.30	4.32
LSMF	75% fish faeces + 25% sugar beet		57.20	2.87	3.27
HSHF	50% fish faeces + 50% sugar beet		66.60	2.45	2.22
SB	100% sugar beet		85.40	1.59	0.13
Experiment-II (mesocosm)					
LCP	Fish feces (animal-based diet)	1:1000	731.30	27.80	45.00
HCP	Fish feces (plant-based diet)		844.30	26.00	21.80
LNP	Feed (brewer’s spent grain)		939.00	40.30	6.24
HNP	Feed (sugar beet expeller)		933.00	17.40	1.38
Experiment-III (pond living lab)					
C	100% wheat	1:2300	383.9	6.69	1.37
F	27% wheat + 73%		379.2	13.61	2.86
P	pellets				

Discussions

Overall findings suggest that managing elemental stoichiometry—by lowering P while increasing sustained-release organic C sources—may help maintain the biofilter function of hybrid pond systems. Also, reducing fish stocks in the outer pond area could optimize the nutritional ecology for zooplankton propagation, controlling algae. Linking interdisciplinary concepts such as ecological stoichiometry theory (C-amendment, P-reduction, but carefully avoiding brownification risk) and a plankton ecology model (fish—scenario), hybrid ponds may become a regenerative form of aquaculture.

Such applications of ecological stoichiometry and planktonic food web ecology in European hybrid pond aquaculture would serve dual purposes: (1) for being EU-water framework directive-compliant in terms of N and P limits, and, (2) possibly “labelling the hybrid pond technology” as a regenerative form of aquaculture under the new EU nature restoration law (Regulation (EU) 2024/1991 of the European Parliament and of the Council of 24 June 2024).

Acknowledgment: The study was funded by the National Agency for Agricultural Research, Czech Republic (project NAZV QL25020009).

INDUSTRIAL DECARBONIZATION WITH SUSTAINABLE PROTEIN PRODUCTION IN THE AT-CZ REGION

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CARMA transforms industrial CO₂ into methane and then into sustainable microbial protein, creating a cross-border innovation chain that connects decarbonization, circular bioeconomy, and resilient feed systems in Austria and Czechia. Inspired by nature, a naturally occurring methanotroph in Central and Eastern European fishponds¹ or municipal sludge², the project would target them to grow on industrial gaseous carbon emissions and naturally generate protein- and nitrogen-rich biomass in the process. In a way, the pollution will become a potential solution. It is already innovative. But the project goes one step further – by answering what to make of that solution? In natural ecosystems such as ponds, these bacteria (methanotrophs) are already part of the fish food web, as in a pond, for example, carp. From that same inspiration, we will characterize and test methanotrophic bacterial proteins (referred to as “C1 gas protein”) for commercial aquaculture.

Below is a comparison of the amino acid profiles of some commercial brands of C1 protein³ for premium carnivorous aquaculture species such as salmon, trout, catfish, or perch, which require a lot of protein and for which sustainable protein sources are in short supply. Most C1 gas protein products available in the market generally exceed the requirements of carnivorous fish but can be of lower quality if not carefully prepared, and may be deficient in either arginine or methionine (Figure 1). Therefore, CARMA products would aim to become competitive by also manipulating manufacturing conditions. Interested in turning CO₂ into sustainable protein? Stay informed about CARMA project results and be part of the CARMA innovation network. Join our stakeholder network and receive project updates, results, and invitations. **Scan the QR code.**

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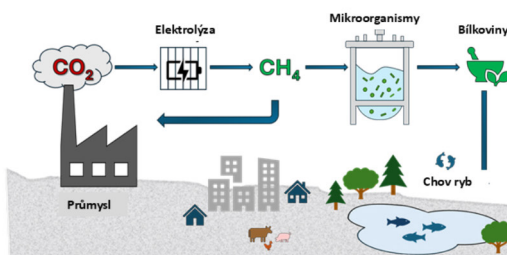
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Evropskou unií



CARMA ATCZ00278

Popis projektu:
CARMA přeměňuje emise CO₂ na metan pomocí elektrolýzy a využívá mikroorganismy k produkci vysoce kvalitní biomasy. Složení živin tohoto alternativního zdroje bílkovin se poté vyhodnocuje a jeho využitelnost jako krmiva testujeme na rybách.

Doba realizace: 01.04.2026 – 31.12.2028
Spolufinancování z EFRR: € 868.200,55



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FAECAL PROPERTIES OF TILAPIA FED TWO DIETS CONTAINING LOW AND HIGH INCLUSION OF LOCB (LOW-OPPORTUNITY-COST-BIOMASS) AT IDENTICAL DIGESTIBLE MACRONUTRIENT-ENERGY INTAKE

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Introduction

Low-opportunity-cost biomass (LOCB) is defined as discarded biomass, plant residues, by-products, and food loss and waste, and typically competes less for land or other natural resources, making it an important inedible feed resource for livestock production. Use of LOCBs in aquafeed is recognized as a tool to lower trophic levels, promote circularity, and limit transgressions of planetary health boundaries caused by intensive aquaculture. Although these systemic advantages of LOCB use are well documented, few studies discuss the problems associated with its use in recirculating aquaculture systems (RAS).

Materials and Methods

Experimental diets and system: Two diets with identical ‘digestible’ feeding values in terms of dry matter (71.5 ± 1.4 g 100 g⁻¹), protein (32.8 ± 0.5 g 100 g⁻¹), carbohydrate excluding fibres (36.9 ± 2.1 g 100 g⁻¹), lipids (3.7 ± 1.0 g 100 g⁻¹), ash (4.5 ± 0.6 g 100 g⁻¹), fibre (0.2 ± 0.2 g 100 g⁻¹), and energy (371.3 ± 3.8 kcal 100 g⁻¹) was fed to mixed-sex Nile tilapia (*Oreochromis niloticus*) housed in a 12 tank Guelph-RAS system (stocking density: 26.7 kg m⁻³, body weight 400–600 g, daily feeding rate 2% of body weight, water temperature 23°C, and dissolved oxygen >6 mg L⁻¹). The low LOCB diet was comprised of 21% LOCB (*food-feed competition free ingredient*: 21% poultry meat and bone meal). The high LOCB diet was comprised of 53% LOCB (*food-feed competition free ingredients*: 10% freshwater fish meal from processing discards, 6% extruded morning cereals factory discards, 21% poultry meat and bone meal, 15% rapeseed expeller meal solvent extracted, 1% freshwater fish oil from processing discards). Each diet was fed in 4 replicate tanks (2 x 4). Another 4 tanks were reserved for a commercial control, *i.e.*, Skretting Standard Expanded, with a similar digestible macronutrient and energy profile. The apparent digestibility was previously determined by employing an endogenous marker, acid-insoluble ash concentration, in feed and feces. The commercial control was placed to verify an industrial standard for fecal particle integrity.

Faeces collection and grading of fecal particles: Each tank was equipped with a Guelph tube, enabling quick, passive passage of feces from the fish tanks to the Guelph tubes via a 45° slope (rise-over-run ratio = 1 vertical:1 horizontal). The bottom of the Guelph tube is a conical silo connected to a screwable receptacle with a tap. A transparent glass bottle (full of water) was screwed to collect fecal particles, which fell freely into the bottle as the fish defecated in the tank and were deposited gently in layers. A vertically stacked seven-stage sieve (diameter 20 cm), generally used for size grading and sorting pond plankton samples (sieve-0= 60 µm, sieve-1= 191 µm, sieve-2= 267 µm, sieve-3= 488 µm, sieve-4= 744 µm, sieve-5= 1285 µm, sieve-6= 2357 µm) was employed to understand particle distribution of faecal particles. Before draining, all sieves were dried by repeatedly applying 2-ply tissue paper from both sides of the sieve. The feces were drained from the top (sieve-6), swirling from the center to the outer diameter in a gentle clockwise motion to spread the samples into a single layer covering the sieve. Then the entire filter unit was gently shaken with a mild flow of tap water, without disturbing the feces, for 1 minute. Then one sieve was removed at a time, and the same process was repeated on the next sieve. The process was repeated till sieve-0 was reached, which had to be scraped by a spoon to release the accumulated water. Sieve 0 was not collected due to extremely low amounts and a high dilution with water. After water was drained from all sieves, the feces from each sieve were scooped off with a spoon, placed in a petri dish, and gently dried overnight at 60 °C for 48 hours. After which, the dry weight of recovered feces per sieve was recorded. Dried fecal particles trapped in the coarsest sieve-6 (2357 µm) were classified as “easily removable”. Whereas fecal particles trapped in sieves 0–5 (60–1285 µm) were classified as “disintegrable”. This classification was further subdivided into two risk categories: breakable feces with a “low risk” (sieve 4–5: >744 µm to <2357 µm) and a “high risk” of re-suspension (sieve 1–3: >191 µm to <744 µm). The measurements were done on a random week, but five days in a row (Monday to Friday) to capture inter-day variability. Before sampling, the fish were acclimatized to the diet and the system for 3 weeks.

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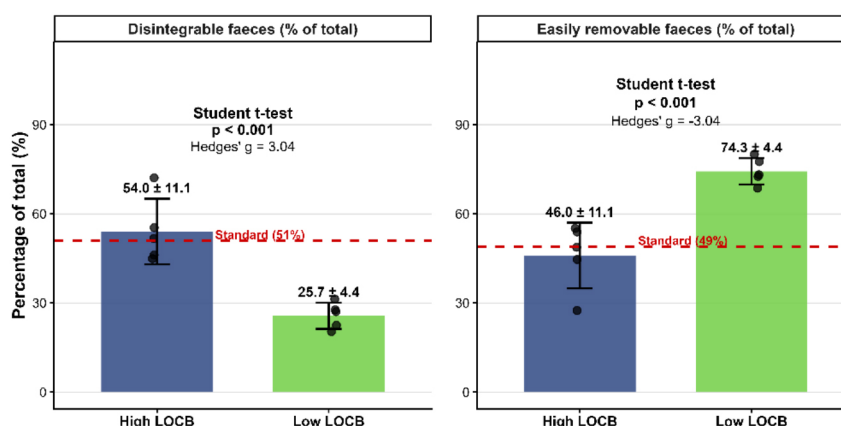


Figure 1: Fecal properties of Nile tilapia fed low and high LOCB feed at identical digestible macronutrient and energy intake. The red line is the commercial control.

Results

High LOCB feed resulted in significantly (Student's t-test, $p < 0.01$) greater proportions of disintegrable feces ($54.0 \pm 11.1\%$ of total feces) compared with Low LOCB feed ($25.7 \pm 4.4\%$ of total feces). Conversely, Low LOCB feed produced significantly greater proportions of easily removable feces ($74.3 \pm 4.4\%$ of total feces) than the high LOCB feed ($46.0 \pm 11.1\%$; $p < 0.01$). The industrial standard of easily removable feces is indicated by the red line in the plot (Figure 1). However, once the fecal particles are disintegrated, the diets do not differ in the share of fecal particles with a high risk of resuspension (Low LOCB feed: $38.1 \pm 1.7\%$; high LOCB feed: $39.7 \pm 8.5\%$ of disintegrable feces), which was not significantly different from the industrial standard.

The very large effect sizes (Hedge's $g = 3.37$) indicate strong separation between groups in terms of influences on fecal particle integrity caused by low (21%) and high (53%) inclusion of LOCBs at almost isogenous digestible macronutrient/energy intake by tilapia.

Discussions

Pushing for greater inclusion of LOCBs in RAS aquafeeds to lower trophic levels, reduce environmental impact, and increase circularity in intensive aquaculture should be undertaken with caution. It may deteriorate the current industrial standards for fish fecal particle integrity, resulting in more stressful on-farm solids management, mechanical drum filter (in) compatibility issues, and preventing clearer water and higher fish welfare in RAS settings. High LOCB feed may satisfy life cycle impact assessment (LCIA) at the systemic level, but can also create *in-situ* problems. Low LOCB aquafeeds may be problematic in LCIA but offer the advantage of cleaner feeds *in-situ* (for RAS). Future research on LOCBs in RAS aquafeeds should focus on resolving this 'sustainability paradox', whereas ponds may address this problem through the food web.

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PREBIOTIC POTENTIAL OF BROWN SEAWEED POLYSACCHARIDES IN *IN VITRO* HINDGUT FERMENTATION MODELS OF FRESHWATER AND MARINE FISH

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Introduction

Disease outbreaks continue to represent a major challenge for aquaculture production, compromising animal welfare and causing economic losses, which highlights the need for alternatives to antibiotics capable of enhancing fish health and resilience to pathogens. Sustainable functional feed additives have gained increasing attention, with brown seaweed-derived polysaccharides emerging as promising candidates due to their immunostimulatory and potential prebiotic properties (Thépot et al., 2021). In this regard, seaweed polysaccharides are not digestible by the fish, suggesting that their beneficial effects may be mediated by the gut microbiota, which ferments these compounds into short-chain fatty acids (SCFAs). In turn, SCFAs may act as mediators of gut epithelial integrity, immune regulation, and host-microbe signalling (Petit et al., 2022; Liu et al., 2023). Despite the proposed microbiota-mediated mechanism, comparative evidence of their direct prebiotic effects across freshwater and marine fish microbiomes remains scarce. Therefore, this study aimed to evaluate the prebiotic potential of laminarin, fucoidan, and alginate using *in vitro* fermentation systems inoculated with microbiota from freshwater Atlantic salmon (*Salmo salar*) and marine European seabass (*Dicentrarchus labrax*).

Materials and Methods

In vitro fermentation bioreactors were set up using faecal material collected by settling from fish reared in recirculating aquaculture systems at Wageningen University (Syropoulou et al., 2024). Four treatments were established in triplicate by supplementing the bioreactors with either no additive (control), laminarin, fucoidan, or alginate from brown seaweeds at a concentration of 5 mg/L. Separate fermentation trials were conducted under the specific rearing conditions for Atlantic salmon (14°C) and European seabass (23°C) and under anoxic conditions for 14 days. Samples were collected periodically for SCFA quantification by gas chromatography, dissolved carbon and nitrogen species quantification, organic matter analyses, and microbial community profiling through V1-V9 16S rRNA gene sequencing with the MinION Mk1C platform (Oxford Nanopore Technologies).

Results and Discussion

The production of SCFAs increased over time in both species, confirming microbial fermentation, while organic matter decreased, likely reflecting substrate consumption for bacterial growth. In Atlantic salmon, the treatment significantly affected the concentrations of propionic, butyric, and caproic acids. Specifically, after 7 days, alginate induced higher butyrate levels than laminarin and the control groups, which further increased by day 14. This pattern may be linked to the higher relative abundance of *Clostridium*, especially *Clostridium beijerinckii*, which ferments complex carbohydrates into butyric acid (Olorunsogbon et al., 2022), whose abundance also increased over time. On the other hand, the most predominant species of the control group at the end of the trial was *Lactococcus lactis*, involved in fermentation of mono- and disaccharides into lactic acid. Ammonium nitrogen (N-NH_4^+) increased over time, indicating microbial mineralization; however, final concentrations were lower in the alginate treatment than in the rest of the experimental groups. Together with the lower total dissolved nitrogen, the decrease in organic carbon, and the concomitant increase in inorganic carbon observed in this treatment, these results suggest that alginate fostered enhanced microbial assimilation, and/or heterotrophic nitrification and subsequent denitrification under oxygen-limited conditions. In European seabass, the treatment effects were more moderate, mainly affecting the levels of valeric, isocaproic, and caproic acids, which were higher in the alginate treatment. Acetic acid concentrations also showed a numerical increase when supplementing the media with alginate, which might be consistent with the slight increase in *Cetobacterium somerae* at day 14 in the media containing alginate compared with the control group, as this species conducts carbohydrate fermentation into acetic acid (Finegold et al., 2003). In addition, the *in vitro* trial of European seabass did show no differences among treatments in dissolved carbon and nitrogen species concentrations, while organic carbon levels increased over time, potentially reflecting bacterial growth and/or SCFA production. Taken together, these findings suggest that alginate promoted the highest fermentative response across both models, supporting its potential as the most effective prebiotic candidate among the tested brown seaweed polysaccharides.

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FROM FRAGMENTED FARM DATA TO INTELLIGENT FEEDING: A MACHINE LEARNING FRAMEWORK FOR INTEGRATED DECISION SUPPORT IN AQUACULTURE

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Feed inefficiency remains a major challenge in salmon aquaculture, leading to economic loss and environmental impact through wasted feed and suboptimal growth. Current feeding practices rely on operator experience and partial observations, while important data is spread across multiple disconnected systems. This makes it difficult to fully understand fish appetite and respond consistently to changing conditions.

In this study, we present a machine learning–driven framework that transforms heterogeneous farm data into a unified basis for feeding decisions. Multiple data sources—including environmental measurements, feeding logs, biomass and welfare indicators, and production records—are integrated into a single analysis-ready dataset. The integration aligns data across site, cage, fish group, and time, with a focus on feeding events. This enables consistent analysis of feeding performance in its biological and environmental context (Fig. 1).

A key contribution is the creation of a structured feature space that captures interactions between fish condition, environment, and farm operations. The dataset includes variables such as fish weight, growth trends, temperature during feeding, and management indicators like days since last treatment. Feature importance analysis shows that feeding behaviour is driven by a combination of factors rather than a single variable (Fig. 2). In particular, fish size, recent growth, and management history play a central role, confirming and extending previous findings from explainable machine learning approaches (Saad et al., 2024). This highlights the need to combine biological, environmental, and operational data when modelling fish appetite.

Based on this dataset, a machine learning framework is developed to support feeding decisions through three stages (Fig. 3). First, a feed prediction model learns current feeding practice from historical data. Second, an outcome model estimates expected growth and feed efficiency (FCR). Third, both models are combined into a simulation layer that explores alternative feeding strategies and evaluates their expected impact. This creates a closed-loop approach where data is used not only for analysis but also to guide future decisions.

Retrospective simulations using real farm data indicate that model-informed feeding adjustments can improve growth performance and reduce feed conversion ratio (FCR) in a large share of cases (Fig. 3). While these results are exploratory, they demonstrate the potential of integrating multi-source data and machine learning to move from reactive feeding toward predictive and adaptive strategies. This represents a shift from experience-based decision making to data-supported optimization under real production conditions.

More broadly, this work highlights a key shift in aquaculture. The challenge is no longer collecting data, but structuring and connecting it across systems. Traditional models rely on simplified relationships between fish size and environmental conditions (Aunsmo et al., 2014; Remen et al., 2016) and do not capture real production dynamics. In contrast, the proposed framework enables learning directly from operational data.

This approach provides a scalable foundation for intelligent feeding systems. It bridges data integration and modelling, enabling future developments such as real-time optimization and autonomous feeding. Ultimately, it supports data-driven feeding decisions for improved performance, reduced waste, and more sustainable production.

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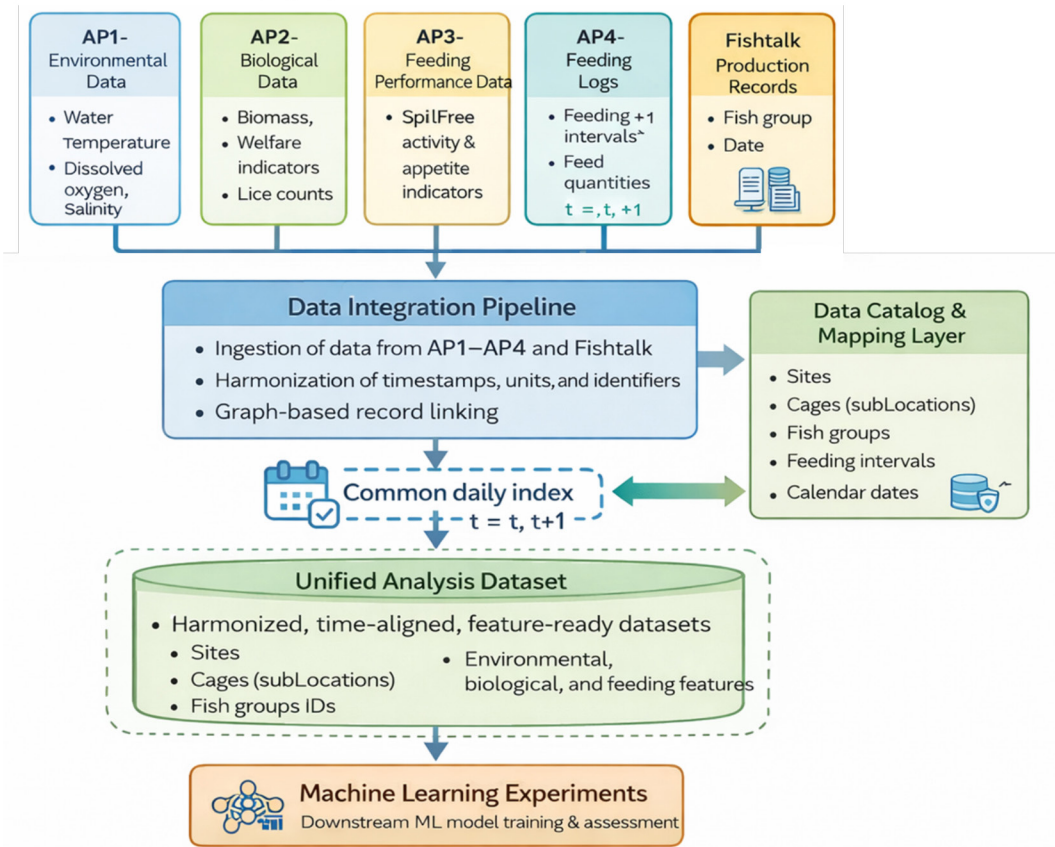


Fig. 1: Integrated data framework showing ingestion, harmonization, and temporal alignment of multi-source farm data into a unified analysis-ready dataset.

Acknowledgements

The work described has received funding from the internal RACE Autoföring project and has been carried out at the ACE facility of SINTEF Ocean. We are also grateful for the contribution of the industry partners, SALMAR, Piscada, and SpillFree in making this project possible.

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EFFECT OF TEMPERATURE ON BIOFLOC DYNAMICS, MICROBIAL COMMUNITIES AND NUTRIENT FATE IN COMMON CARP CULTURE (*Cyprinus carpio*)

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Introduction

Biofloc technology (BFT) is an alternative aquaculture approach that relies on microbial aggregates to improve water quality and potentially provides an additional nutritious food source for cultured fish. Although BFT is usually applied under tropical conditions, typically between 25 and 30°C, its functioning at lower temperatures remains poorly understood. Nevertheless, the development of BFT under temperate continental conditions could offer promising opportunities for the diversification of inland aquaculture in Europe. The present study investigated the effect of four rearing temperatures (15, 20, 25 and 30°C) on biofloc dynamics, nutrient dynamics and fish performance using common carp (*Cyprinus carpio*) as the culture model.

Material and methods

Experiment was conducted on juvenile common carp (initial mean body of 15 g) in 100l tanks in triplicate at an initial stocking density of 5 kg m⁻³. The experiment consisted of three successive phases. A first 4-week stabilization period was carried out in order to allow the stabilization of the biofloc which was inoculated from a stock solution at 23°C. Floc is considered stable when floc volume exceeded 15 mL L⁻¹, TAN concentration remained below 2 mg L⁻¹ and nitrite concentration below 1 mg L⁻¹. A second 3-week period was applied to gradually reach the experimental temperature (15, 20, 25 or 30°C). Then the 12-week experimental phase was conducted. Feed ratios (15°C : 1.5%, 20°C : 1.5%, 25°C : 3%, 30°C : 3.5%) were adjusted daily according to tank biomass and water temperature.

Water quality and biofloc development were daily or biweekly monitored by measuring temperature, dissolved oxygen, floc volume, pH, conductivity, alkalinity, TAN, nitrite, nitrate and suspended solids. Fish feed, molasses, sodium bicarbonate and water renewal were quantified. Total carp biomass was measured four times: at stocking (before the stabilization phase), at the start of the experimental phase (T0), after 6 weeks, and after 12 weeks (end of the experiment). Individual weight and length of 20 fish per tank were recorded every two weeks.

Carbon and nitrogen budgets were then established to determine how the nutrients introduced through feed and molasses were partitioned within the system, to identify where carbon and nitrogen accumulated, and to assess whether carp consumed biofloc particles in addition to commercial feed. Total carbon and nitrogen were measured by isotopic analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) in feed, fish muscle, and in both the liquid and solid fractions of the biofloc collected from Imhoff cones after 30 minutes of decantation.

Parallely, microbial community associated with the biofloc (rotifers, ciliates, ...) was identified and quantified. Floc particle size were characterized microscopically through measurements of particle area and perimeter.

	FCR	SGR (%/day)	Floc volume (mL/L)	TAN (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)
15°C	0.90	1.34	15	2.2	1.0	384.3
20°C	0.84*	1.58*	21*	2.0*	0.1*	557.0*
25°C	1.00**	2.09**	32**	3.2**	0.4**	600.2**
30°C	1.14	1.76	26	2.3	0.2	616.8

Table 1 : mean FCR, SGR, biofloc volume and water quality Nitrogen parameters during the 12-week experimental phase at 15, 20, 25 and 30°C. Values are means per treatment; asterisks indicate reduced replication (* single replicate, ** two replicates)

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Results

Preliminary results showed that the specific growth rate (SGR) increased with temperature, reaching 0.90% day⁻¹ at 15°C, 0.84% day⁻¹ at 20°C, 1.00% day⁻¹ at 25°C and 1.14% day⁻¹ at 30°C. In contrast, feed conversion ratio (FCR) was not improved with increasing temperature. The best feed conversion ratio was observed at 15°C (1.34), whereas higher values were recorded at 20°C (1.58), 25°C (2.09) and 30°C (1.76). However, the results obtained at 20°C and 25°C should be interpreted with caution, as they were based on a single replicate and two replicates, respectively. Two tanks at 20°C and one tank at 25°C were excluded from the analysis due to a bacterial disease affecting the carp and altering the experimental results.

We observed that biofloc-related parameters were strongly influenced by temperature. Mean floc volume remained lower at 15°C (15 mL L⁻¹) compared to other temperatures with mean values ranging from 21 to 32 mL L⁻¹ (Table 1). TAN concentrations remained relatively stable among treatments, ranging from 2.0 to 3.2 mg L⁻¹. Nitrite concentrations were consistently higher in the 15°C tanks throughout the experiment (mean value: 1.0 mg L⁻¹), compared with only 0.1–0.4 mg L⁻¹ at higher temperatures. Moreover, nitrate concentrations increased with temperature, from a mean value of 384.3 mg L⁻¹ at 15°C to 557.0, 600.2 and 616.8 mg L⁻¹ at 20, 25 and 30°C, respectively.

Additional analyses are currently in progress to further characterize the functioning of the system. Carbon and nitrogen contents in fish muscle samples are still under investigation in order to complete the nutrient budgets and better understand the contribution of biofloc to fish nutrition. Likewise, the identification and quantification of microorganisms associated with the biofloc, including rotifers, ciliates and other microbial groups are ongoing. Measurements of floc particle area and perimeter are also underway and should provide further insight into how temperature affects biofloc structure and composition.

Overall, our results show that biofloc technology (BFT) remained efficient at temperatures lower than those usually reported for this aquaculture rearing system (25–30°C), while maintaining stable water quality and acting effectively as a biological treatment system. Common carp exhibited satisfactory growth performance under all tested conditions, demonstrating good adaptability to BFT across the evaluated thermal range. These findings suggest that BFT could represent a promising diversification strategy for inland aquaculture in continental Europe, particularly under temperate conditions.

MODULATORY EFFECTS OF BREWER'S SPENT GRAIN COMPONENTS ON PERISTALSIS IN THE ATLANTIC SALMON (*Salmo salar*) INTESTINE

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Introduction

Utilizing agricultural and industrial side streams offers a pathway to more sustainable salmon feed. While fisheries and aquaculture by-products are nutritionally valuable, agricultural side streams can serve as viable alternatives to traditional ingredients like soy protein concentrate (SPC) and gluten. Brewer's spent grain (BSG), the primary by-product of the brewing process, offers a potential sustainable solution for aquaculture feeds. Approximately 3.4 million tons of BSG are generated annually in the EU, with Germany accounting for about 2 million tons (Steiner et al., 2015). Although the brewing process extracts starch, leaving residual lipids, fibers, and proteins, dried BSG contains only 20–30% protein (Karlsen and Skov, 2022), too low for significant inclusion in salmon diets. Further processing can yield in a high-protein BSG concentrate (HP-BSG, ~50% protein) possibly suitability in salmon feed. However, HP-BSG retains a high non-starch polysaccharide (NSP) content of approximately 26%. Dietary fiber fractions can affect intestinal peristalsis, as previously shown in ballan wrasse (Le et al., 2021). Consequently, this project aimed to analyze how HP-BSG and specific fibers (β -glucans and arabinoxylans) modulate peristalsis in the Atlantic salmon intestine.

Material and Method

Boluses of fish meal (FM), HP-BSG and SPC as well as the NSPs arabinoxylan and β -glucan were subsequently introduced into an *ex vivo* intestine preparation (n=6 per treatment) mounted in a Ringer's solution bath. The intestinal movements were recorded, and changes in intestinal shape analyzed over time using in-house software. Peristaltic patterns are visualized in a heatmap and were identified and counted visually. Further, the time of bolus passage was recorded. Differences in contraction frequency between treatments were analyzed using anova or non-parametric alternatives. A Kaplan-Maier equation was applied to bolus passage times with log-rank test to compare between treatments.

Results and Discussion

When comparing the passage rates of HP-BSG, SPC, and FM boli no significant differences between treatments were found (log-rank test, $p = 0.23$). Passage time is dictated by gut contractions, which can travel in a retrograde (slowing passage) or anterograde (accelerating passage) direction (Le et al., 2019). In both midgut and hindgut, anterograde and reterograde contraction frequency remained unaffected by bolus type (1-w aov, $p = 0.95$).

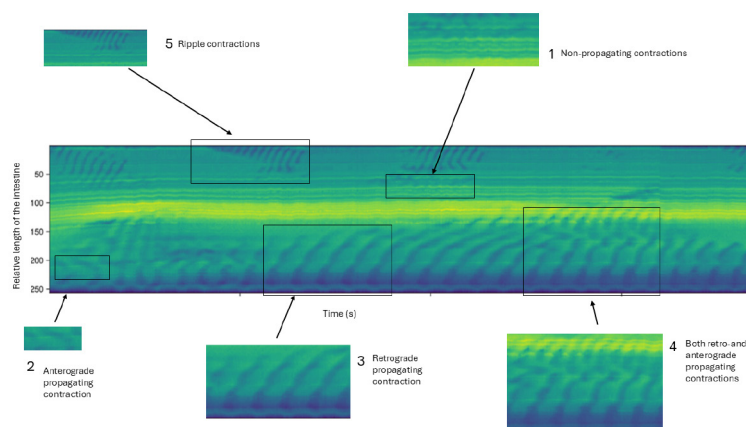


Figure: Heatmap showing the different peristaltic patterns

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However, comparing the effects of NSPs to FM yielded unexpected results with β -glucan exhibiting a longer passage time than FM, with arabinoxylan in-between (KLM, $p < 0.01$). This contrasts findings in ballan wrasse, where nutrient-rich boli (protein and lipid) were retained by retrograde peristalsis, resulting in longer passage times than cellulose boli (Le et al., 2021). In the current study, NSP boli did not affect retrograde contractions in the hindgut ($p = 0.407$). Anterograde contractions increased in the midgut with FM compared to β -glucans or arabinoxylans (Kruskal-Wallis test, $p = 0.001$; FM vs AX, $p = 0.003$; FM vs BG, $p = 0.004$). No bolus effects were observed in the hindgut. Bolus composition did not affect contraction length ($p > 0.05$).

Overall, the peristaltic braking mechanism of slowing passage rates in response to nutrients was not observed in the A. salmon intestine. Conversely, FM passed faster than β -glucan. These findings suggest that in Atlantic salmon the physical attributes of the bolus may exert a greater impact on peristaltic mechanisms than its chemical composition.

Acknowledgment

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POLICY INDICATORS FOR FISH WELFARE IN EUROPEAN AQUACULTURE: A HARMONISED FRAMEWORK FOR OUTCOME-BASED ASSESSMENT

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Rationale

Fish are sentient beings capable of experiencing pain and distress (Lambert et al. 2022), yet the European Union (EU) currently lacks a practical, standardised set of welfare indicators for the approximately 640 million fish slaughtered annually (Mood et al., 2023). Current legislative frameworks often rely on outdated input-based standards that fail to reflect recent scientific advancements. This presentation draws on a recently published report (Eurogroup for Animals, 2026), which proposes three core policy indicators designed to be measurable, auditable, and applicable across major production systems to support regulatory compliance and funding conditionality.

Proposed Indicators

The framework identifies three priority indicators to capture acute risk, chronic compromise, and retrospective system-level failure:

1. **Dissolved Oxygen (DO) Stability (Resource-based Indicator):** DO is a critical preventative indicator. Maintaining stability enables proactive risk reduction before the onset of suffering. A minimum concentration of 70% is considered essential for farmed fish welfare.
2. **External Injuries and Fin Damage (Animal-based Indicator):** These provide a cumulative record of suboptimal environmental and husbandry conditions. Scoring can be integrated into existing post-slaughter inspection protocols under Regulation (EU) 2017/625 to minimise additional handling stress.
3. **Mortality (Animal-based Indicator):** Mortality serves as a retrospective signal of severe welfare breakdown. Standardising mortality data across the EU would allow for benchmarking and the identification of systemic risks.

Policy Relevance and Discussion

These indicators align with key EU instruments, including the Strategic Aquaculture Guidelines (SAG), the Common Fisheries Policy (CFP), and the European Maritime, Fisheries and Aquaculture Fund (EMFAF). By moving beyond mere input-based requirements toward outcome-based assessment, this framework provides a transparent mechanism for monitoring and enforcement. Just as the modernisation of terrestrial welfare rules strengthens the resilience of EU agriculture, establishing specific, science-based rules for aquatic animals is a strategic investment in the sustainability and fairness of the aquaculture sector. Future directions should also integrate automated behavioural monitoring, such as respiratory and swimming patterns, as these technologies become commercially viable, as they provide direct, outcome-based indicators of fish welfare.

Acknowledgment

This work was supported by Eurogroup for Animals.

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EFFECT OF DNase TREATMENT ON THE POST-THAW MOTILITY AND FERTILIZING CAPACITY OF CRYOPRESERVED COMMON CARP (*Cyprinus carpio*) SPERM

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Introduction

Cryopreservation is no longer just a laboratory technique. It has become essential for modern carp farming (Judycka et al., 2024). Over the past decades, numerous cryopreservation protocols have been proposed for common carp sperm (*Cyprinus carpio*), but a fully standardized method is still lacking (Sotnikov et al., 2024). The present study aimed to develop a unified cryopreservation protocol for common carp sperm by testing DNase concentrations (0, 10, 20, 50, 100 µg), and evaluating post-thaw motility using computer-assisted sperm analysis (CASA). Results show that an optimized DNase treatment can maintain or improve sperm motility after thawing. It suggests that a single, standardized protocol is feasible for common carp. This approach could support the use of cryopreserved sperm in commercial carp farming.

Materials and Methods

Sperm samples were collected from *Cyprinus carpio* broodstock maintained for three generations in a recirculating aquaculture system, ensuring adaptation to closed conditions. Milt was obtained by gentle abdominal stripping, collected in Falcon tubes, and kept on ice until analysis. A carp extender (200 mM glucose, 40 mM KCl, 30 mM Tris, pH 8.0) was used as the immobilizing medium. The activating solution contained 45 mM NaCl, 5 mM KCl, and 30 mM Tris (pH 8.0) with 1% BSA. Sperm was diluted 1:1 (0.5–2 × 10⁹ cells/ml), mixed with 0.5 ml of 10% methanol as cryoprotectant and 4 ml of extender, and supplemented with DNase at 0, 10, 20, 50, or 100 µg. The mixtures were loaded into 0.5 ml straws, cooled in liquid nitrogen vapor for about 3 minutes, and then thawed at 40 °C for 13 seconds. Motility after thawing was assessed using CASA. Measurements were first made on non-activated sperm (2 µl in a Makler chamber), then after activation by adding 9 µl of activating solution with BSA and 1 µl of thawed sperm. Statistical analyses were performed using Jamovi (version 2.6.44.0), with a significance level set at 0.05.

Results and Discussion

Across the three individuals (Figures 1 and 2), DNase concentration did not affect progressive motility before activation in Fish 1 and Fish 3 ($p > 0.05$). This agrees with the microscopic observations, which showed low motility, jelly formation, and limited sperm activity in the non-activated state. In contrast, Fish 2 showed a significant response to DNase without activation ($p = 0.003$), suggesting that DNase improved motility, likely by reducing clumping and improving cell dispersion. After activation, a significant effect of DNase was observed only in Fish 1 ($p = 0.043$). Fish 2 ($p = 0.054$) and Fish 3 ($p = 0.169$) did not show significant changes, despite visible differences in concentration, clumping, and cell integrity. Overall, the best motility and microscopic quality, including reduced jelly, fewer clumps, and fewer dead cells, were observed at 10–20 µg DNase. Higher concentrations (50–100 µg) were often associated with more dead cells, suggesting a harmful effect at high doses. Therefore, 10–20 µg appears to be an appropriate range for common carp sperm cryopreservation, balancing motility improvement and cell integrity.

Acknowledgment

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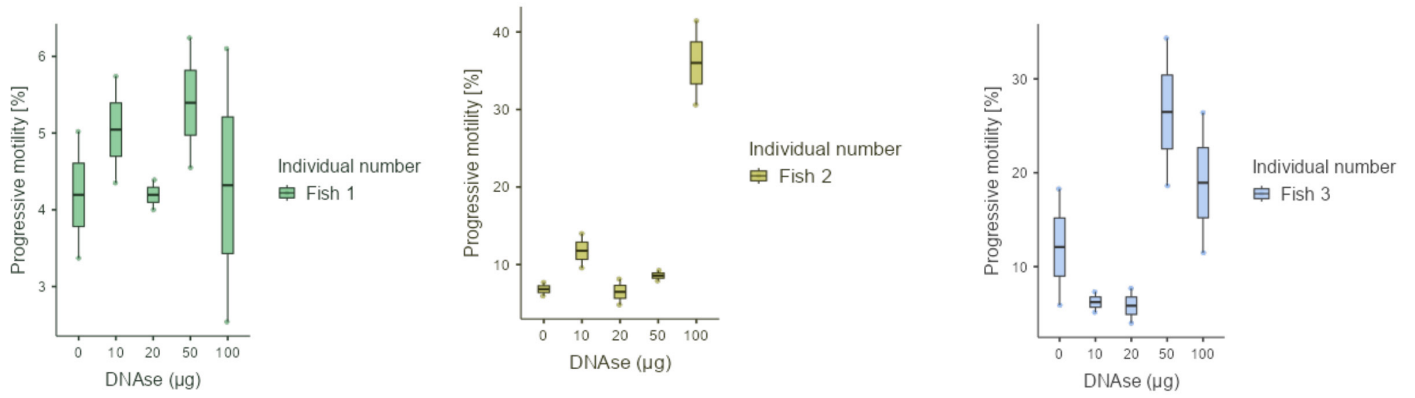


Figure 1: Progressive motility of the three fish sperm after DNase treatment without activating solution.

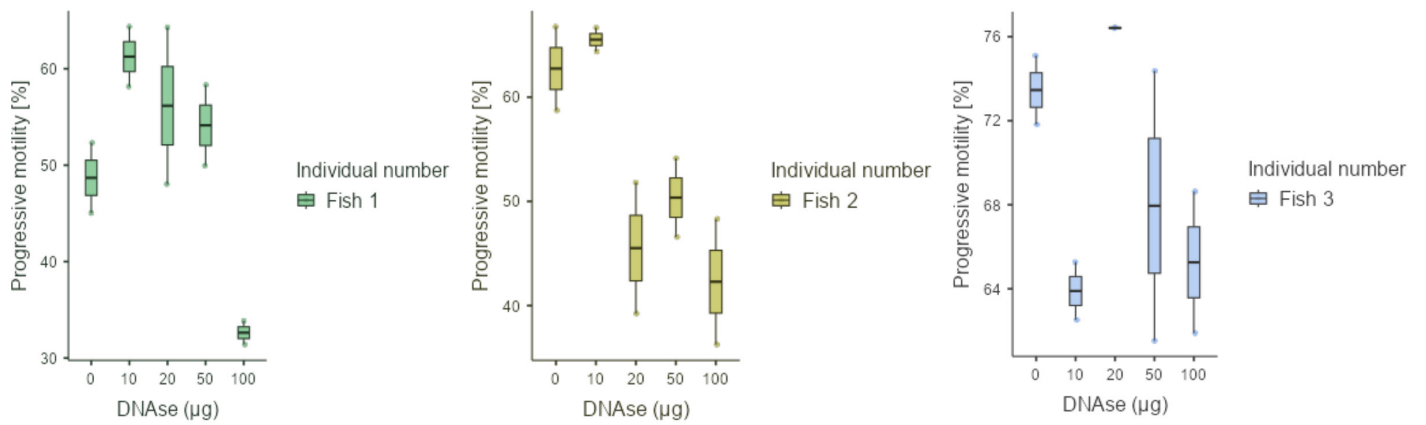


Figure 2: Progressive motility of the three fish sperm after DNase treatment with activating solution

THE COLLAPSE OF *Chamelea gallina* IN THE NORTHERN ADRIATIC: INSIGHTS FROM A BIOENERGETIC MODEL

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Introduction

Chamelea gallina represents an economically and ecologically important species in the Adriatic basin, accounting for over 98% of 'venus clams' of the Italian production. In the last decades, however, the harvest has been steadily decreasing: According to Romanelli, 2009, *C. gallina* landings dropped from about 100,000 to 21,000 tonnes/year in 2005. Since then, landings have been fluctuating, due to a combination of changing environmental factors, fishery regulations and management activities, carried out by Consortia for the Management of this resource (CO.Ge.Vo). Such activities include stock assessment, based on yearly sampling along transect in fishery area, and juvenile relocation: to same extent, *C. gallina* is therefore being managed in a culture based fishery regime. According to (Grazioli&al., 2022), climate change related increase in summer water temperature, heat waves, extreme events leading to massive discharge of debris and sediment from the rivers might have contributed to the clams massive mortalities observed in several Northern Adriatic areas. However, the role of the steadily decrease in the trophic level of Northern Adriatic may also be playing a role in determining the collapse of *C. gallina* population observed in 2024 and 2025 off the coast of Venice and Chioggia, which caused the complete stop of the fishery. In this paper, we used an individual bioenergetic model to investigate the potential role of energy limitation in reducing both growth rate and energy investment in reproduction of this species.

Materials and methods

The bioenergetic model, is based on the formulation used in (Bertolini&al., 2021) to simulate the individual growth of *Ruditapes philippinarum*. The two most important parameters, namely the Clearance Rate, CR, and the Respiration Rate, RR, of a standard animal (1g wet weight) at optimal temperature, were determined in a nine months long experiment, in which 300 specimens of *C. gallina* were kept in a land-based raceway, previously used for *R. philippinarum* prefattening, connected to a shallow water lagoon located in the Po River Delta. The trial started in October 2024 and ended in June 2025. The raceway bottom was covered with sediment. Salinity ranged from 20 to 24 psu and temperature from 3 °C to 28 °C. Mortality rate was low during the first 6 months but rapidly increased in May and June, probably due to higher water temperatures than those usually experienced by this species. Specimens were sampled with approximately monthly frequency from October 2024 to May 2025. Besides the biometric parameters (total length, width, thickness, total weight, fresh and dry soft body weight, fresh and dry shell weight), the clearance rate and the respiration rate were determined through dedicated experiments in November 2024, February 2025 and May 2025. Respiration and clearance rates were normalized on correspondent dry weights. The effect of water temperature on clearance rate and respiration was taken into account based on the bell-shaped function used in (Bertolini&al., 2021). The clearance rate showed a higher variability, compared to respiration one. The median values of RR of the three data sets, 0.76 mg_{O2} g_{dw}⁻¹ h⁻¹ and the median CR determined in February 2025, 1.39 L g_{dw}⁻¹ h⁻¹, were in accordance with previous literature estimates. Considering that the clams were kept in a non-natural environment, the model application employed an estimate of CR = 2.28 L g_{dw}⁻¹ h⁻¹ based on the third quartile value of the February distribution, assuming that clams would perform better in their natural environment, compared with the mesocosm trial.

Results and discussion

The bioenergetic model was used to simulate clam growth at a sampling site regularly monitored by Venice and Chioggia Co.Ge.Vo. The model was forced by water temperature and Chlorophyll a concentration, which is taken as a proxy of feeding particles. Time series of daily values of both inputs were downloaded from Copernicus Marine Service from September 2022 to December 2023: their evolution is shown in **Fig. 1a**. To test the potential effect of energy limitation, the growth of a juvenile individual was simulated. The results, shown in **Fig. 1b** shows that the growth is limited, in particular during the summer 2023 in which the concentration of chlorophyll-a was found to be, on average, around 0.001 mg L⁻¹. This preliminary finding suggests that the negative trend in primary productivity observed in the North Adriatic in the last

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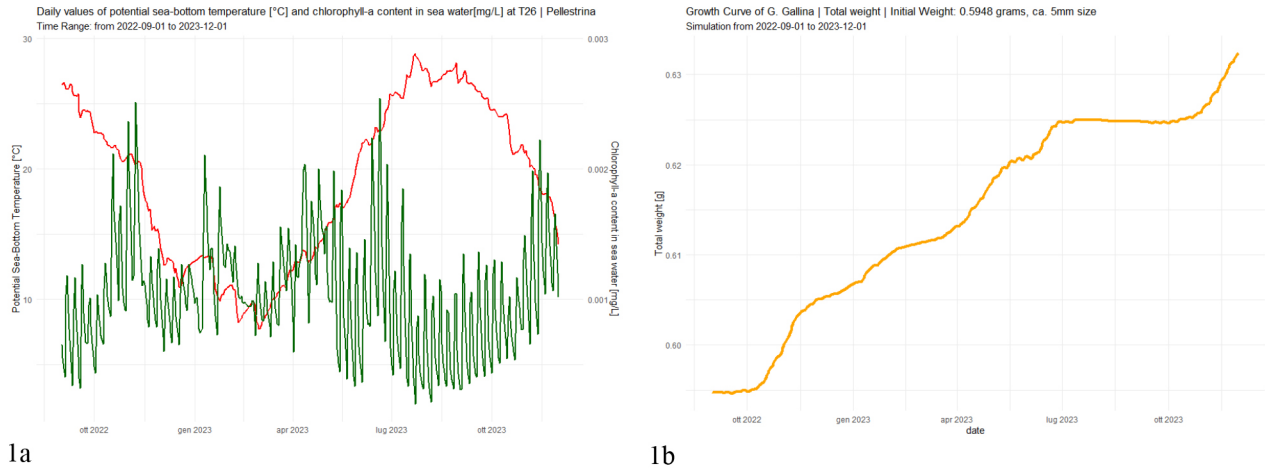


Figure 1: 1a) Time-series (September 2022- December 2023) of water temperature at sea-bed level [°C] and concentration of chlorophyll- a [mg L⁻¹] at a site monitored by Venice and Chioggia CoGeVo. The summer/early autumn 2023 was characterized by high temperatures and low chlorophyll-a concentration. 1b) simulated individual growth in terms of total fresh weight [g] of *C. Gallina*, initial length 5mm.

decades (Giani&al. 2012) could be added to the list of causes of the decreasing trend and the slow recovery after mass mortalities of *C. gallina* abundances. However, microphytobenthos should also be considered as a potential energy source, which could be correlated to water transparency: this aspect could be crucial and should be investigated to get more insights on the collapse of the population and the recovery rate.

Acknowledgement

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AQUAPONIC INTEGRATION OF OREOCHROMIS NILOTICUS AND ALLIUM CEPA: EFFECTS ON WATER QUALITY, FISH PERFORMANCE AND PLANT PRODUCTION

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Introduction

The current study assessed the performance of Nile tilapia (*Oreochromis niloticus*) integrated with green onion (*Allium cepa* L.) in a deep-water culture (DWC) aquaponic system in terms of water quality fish growth hematological and biochemical responses and plant production.

Two identical aquaponic units (AQ) were used while conventional fish tanks (CF) served as the control. Juvenile Nile tilapia with an initial mean weight of 29.77 g were stocked at a density of 50 fish per tank and fed with a commercial feed (32% crude protein) for 40 days (two consecutive plant production cycles) (Figure 1).

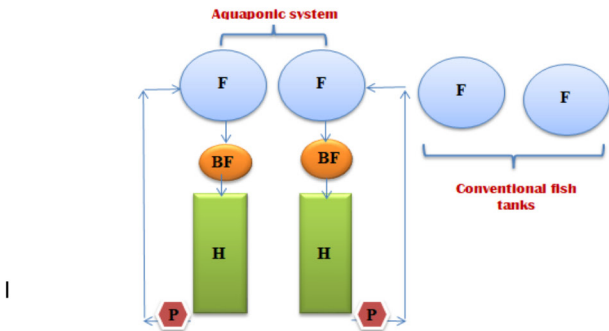


Figure 1. Schematic diagram of the experimental setup. The aquaponic system consisted of two identical units. Each aquaponic unit comprised (F) a fish tank, (BF) a mechanical and biological filter, (H) a hydroponic unit, and (P) a pump. Conventional fish tanks were maintained alongside the aquaponic system and were identical to the fish tanks used in each aquaponic unit.

Results indicated significant improvement in water quality of AQ with lower concentrations of ammonia, nitrite, and phosphate compared with CF (Table 1). alkalinity and pH levels were lower in hydroponic units and the biological filter than in fish tanks for both AQ and CF. Fish reared in AQ showed significantly higher final weight weight gain specific growth rate and protein efficiency ratio and lower feed conversion ratio compared to CF (Table 2). For plant production, Aquaponic significantly enhanced onion vegetative growth. Total chlorophyll content was also significantly higher in Aquaponic indicating improved photosynthetic capacity. While bulb number remained comparable between the two systems. Thus this study shows that the aquaponic system is a better rearing environment for Nile tilapia with the added advantage of plant production nutrient recycling and lower environmental impacts.

Table 1. Water quality parameters in a DWC aquaponic system integrating Nile tilapia (*Oreochromis niloticus*) and green onion (*Allium cepa* L.) and in conventional fish tanks

Parameter	hydroponic units	Biological filter	Aquaponic fish tanks	Conventional fish tanks
Ammonia (mg/L)	0.13 ± 0.02 ^d	0.18 ± 0.05 ^c	0.53 ± 0.13 ^a	0.80 ± 0.10 ^a
Nitrite (mg/L)	0.08 ± 0.03 ^d	0.35 ± 0.08 ^c	0.57 ± 0.06 ^b	0.70 ± 0.10 ^a
Nitrate (mg/L)	31.6 ± 8.4 ^b	123.1 ± 11.8 ^a	20.6 ± 5.0 ^c	23.7 ± 3.8 ^c
Phosphate (mg/L)	1.77 ± 0.46 ^b	4.25 ± 0.05 ^a	2.60 ± 1.15 ^{ab}	2.00 ± 0.00 ^a
pH	7.20 ± 0.10 ^c	7.30 ± 0.10 ^c	7.67 ± 0.06 ^b	7.80 ± 0.00 ^a
Alkalinity (mg/L as CaCO ₃)	127.3 ± 2.5 ^c	146.0 ± 1.0 ^b	156.0 ± 1.0 ^a	155.0 ± 1.0 ^a

Values are presented as mean ± SD. Different superscript letters within the same row indicate significant differences among compartments (one-way ANOVA followed by Duncan's multiple range test; $p < 0.05$).

Table 2. Growth performance and feed utilization of Nile tilapia (*O. niloticus*) reared in aquaponic and conventional systems.

Parameter	AQ	CF	p-value
IW (g)	30.10 ± 0.27	29.57 ± 0.20	0.165
FW (g)	46.29 ± 0.81	39.99 ± 0.39	0.027
WG (g)	16.19 ± 1.07	10.42 ± 0.59	0.040
SGR (% day ⁻¹)	0.65 ± 0.04	0.42 ± 0.02	0.040
DWG (g day ⁻¹)	1.72 ± 0.10	1.21 ± 0.07	0.041
FI (g)	18.97 ± 0.61	16.23 ± 0.75	0.061
FCR	1.18 ± 0.04	1.56 ± 0.01	0.021
PER	3.05 ± 0.09	2.20 ± 0.04	0.032

Values are presented as mean ± SD. Different superscript letters within the same row indicate significant differences among compartments (one-way ANOVA followed by Duncan's multiple range test; $p < 0.05$).

MOULT FINAL ABDOMINAL SEGMENT WIDTH: A NON-INVASIVE APPROACH TO ASSESS SHRIMP LARVAL GROWTH IN LIVE PREY FEEDING TRIALS

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Introduction

Aquaculture is an industry providing animal protein to meet the needs of a growing world population (FAO, 2022). In marine shrimp production, nutrition remains a major limiting factor in hatcheries sustainability by affecting growth, quality, survival rate and post-larvae production (Campillo, 1979; Sorgeloos et al., 1998). The common shrimp, *Palaemon serratus* is widely distributed along European and North African coasts (Huxley, 2013). Despite early aquaculture trials (Campillo, 1979; Rodriguez, 1981) raised in the laboratory, were stocked in salt ponds of the province of Cádiz (SW coast of Spain, it is not currently farmed. However, it remains a promising candidate for European aquaculture. This study investigates larval performance of *P. serratus* under controlled conditions using different feeding regimes including a local rotifer species (*Hexarthra* sp.). The low larval density in our experimental tanks constrained direct sampling, leading us to investigate a non-invasive growth monitoring approach based on the daily collection of moults at the water surface.

Materials and methods

One ovigerous female *P. serratus* was isolated from our broodstock. Upon hatching, larvae were reared in 10 L Zug bottles under three feeding treatments, each in triplicate. To assess the effect of diet on development, growth and survival, we tested the following three diets: *Artemia* nauplii (**A**), a mixed diet (50% *Hexarthra*, 50% *Artemia*) (**RA**), and a final diet in which larvae were first fed *Hexarthra* sp. during the first five days post-hatching, followed by a mixed diet (50% *Artemia*, 50% *Hexarthra*) for 2 days, and finally fed on *Artemia* nauplii exclusively until the end of the experiment (**RTA**).

Moults were collected daily, the total number of one morphologically recognizable part (tail-fan) were counted. Developmental stages were assigned to each moult. Tail fans from each tank were photographed (**Figure 1**) on a “Cellule de Nageotte” with 0.25mm grid lines used as the size reference and measurements were performed using ImageJ software.

Results

Feeding regimes had a highly significant effect on final abdominal segment width at different developmental stages (**Figure 2**), where the *Artemia* (**A**) treatment resulted in a wider last abdominal part than the **RTA** treatment until stage 4 ($P < 0.001$). The mixed diet (**RA**) ranked second place with significantly greater values than **RTA** treatment at stage 4 and 6 ($P < 0.01$).

These results highlight additional metrics, including daily moult counts and biometric measurements performed at the end of the experiment, after metamorphosis into post-larvae, using the conventional invasive method, which involves measuring and weighing sacrificed post-larvae. Final length and weight showed the same pattern as the non-invasive approach, with significantly higher values in treatment **A** than in treatment **RA** ($P < 0.05$). Larvae in treatment **A** also had a faster development up to stage 4 and exhibited higher moult counts per stage.

Discussion

Zootechnical performance results are consistent with those obtained from measurements of the final abdominal segment width, confirming that the larvae fed exclusively on *Artemia* nauplii exhibited overall higher performance than the other treatments. These findings suggest that rotifers of the genus *Hexarthra* are not suitable for shrimp larval rearing. This may be explained by their evasive behavior (Kak and Rao, 1998) *Ceriodaphnia cornuta*, which has also been reported in fish larvae, where *Hexarthra* sp. are actively avoided when alternative prey are available (Mookerji and Rao, 1993).

This study also demonstrates the feasibility of monitoring growth through the daily collection of moults. This non-invasive approach avoids the stress induced by larval capture, which can increase energy demand and negatively affect biological performance (Martínez-Antonio et al., 2019) decreasing shrimp epidemics and water pollution in some coastal areas. However, salinity levels outside the optimal range could impose several physiological constraints that would in turn affect growth and survival, particularly in the presence of additional stressors (e.g. high densities, handling practices, and hypoxia). At an industrial scale, automated image-based measurement of these moults could provide an efficient tool to monitor growth and zootechnical performance in shrimp hatcheries.

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Figures

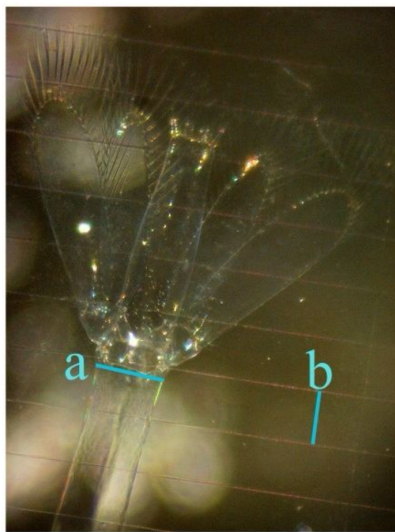


Figure 1: Last abdominal segment width measurement method

a: abdomen width b: Reference size

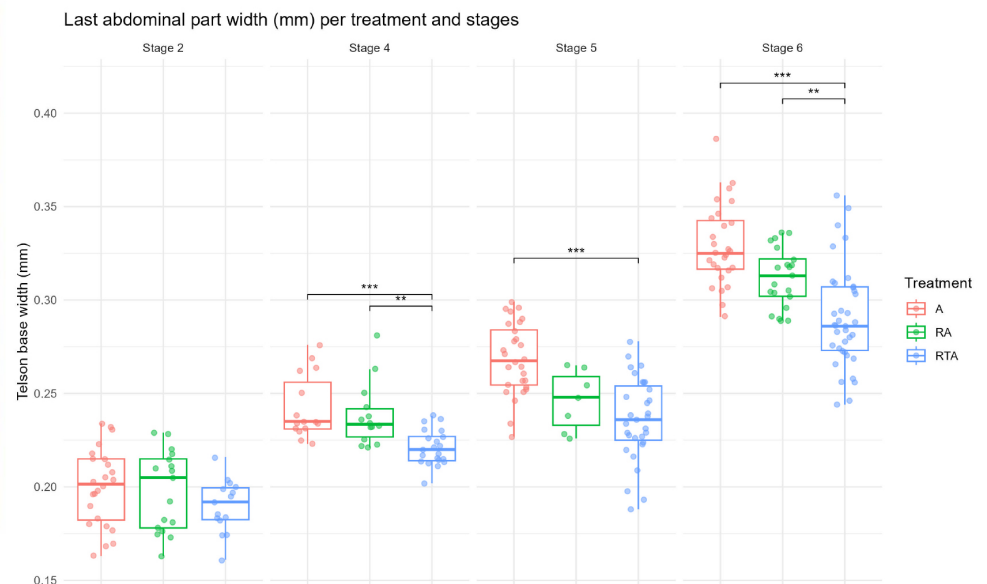


Figure 2: Last abdominal segment width measurement (mm) per treatment and stages. *** = $P < 0.001$, ** = $P < 0.01$

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FROM INDICATORS TO ENGAGEMENT: LESSONS FROM VERIFISH ON COMMUNICATING SEAFOOD SUSTAINABILITY

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Introduction

Communicating seafood sustainability to non-specialist audiences remains difficult because the subject is complex and often fragmented across environmental, nutritional, socio-economic, biodiversity, and provenance-related dimensions. In practice, this complexity is often communicated either through technical language that excludes general audiences or through simplified claims that remove context and credibility. The Horizon Europe project VeriFish was developed to address this gap by creating a verifiable indicator framework for seafood and translating it into communication tools that could support informed understanding and responsible choices. The project combined work on indicators, scoring logic, communication guidelines, a web application, factsheets, media products, and outreach campaigns, with the aim of making sustainability-related information easier to understand and use across different audiences. The framework was built around a simplified Driver-Pressure-State-Impact-Response logic and included environmental, nutrition and health, socio-economic, and provenance dimensions. Eurofish also led the development of guidelines for how to visualise and communicate these indicators in ways that remain accurate while understandable to consumers and other value-chain actors.

A central premise of VeriFish was that communication should not be treated as a final dissemination layer added after technical work is complete. Instead, communication needed to be integrated into the design of outputs from the beginning, including format, wording, level of detail, and visual framing. This approach was embedded in Work Package 3, which aimed to transform VeriFish seafood and aquaculture indicators into accessible media products, build a community of practice around those products, and test their effectiveness through outreach campaigns and stakeholder engagement. Planned outputs included digital factsheets, flashcard-based educational games, puzzles, storytelling sessions, short videos, newsletters, articles, and a web app serving as the main interface for structured information. To support this work, VeriFish included collaborative workshops and user-oriented discussions involving seafood producers and consumer representatives. The project description envisaged at least three design and requirements workshops to ensure that products responded to real user needs rather than internal project assumptions. In practice, four workshops were organised, with around 25 participants per workshop. Across the project, around 30 videos were produced, one web app was created integrating more than 100 species, and factsheet-style outputs could be extracted from the app. Dissemination was reinforced through social media channels and through the final conference, which gathered around 50 participants in person and 35 online.

Results and Discussion

The VeriFish experience showed that communication products were most effective when they were visually clear, concise, and adapted to the way audiences actually engage with information. Among the products developed, the card games worked especially well because they were easy to understand, fast to use, and visually engaging. This is consistent with the original media-product design, which explicitly included flashcard and puzzle-style formats for children and citizens as a way to communicate sustainability, nutrition, and biodiversity-related information in a more approachable manner. The project web app served a different but equally important role: rather than competing for attention through simplification alone, it functioned as the main structured information hub, capable of holding more detailed content while still presenting it in a navigable and user-oriented format. This division between lightweight entry products and a deeper information source proved effective.

A second clear lesson concerned text density and framing. Stakeholder exchanges repeatedly showed that general audiences need attractive framing and visual support to stay engaged. High text density discouraged interaction and made information appear difficult, even when the content itself was relevant. This forced a discipline of precision: the more information that was included, the more concrete and economical the wording had to become. A third lesson concerned video communication. Short videos attracted attention, while longer formats lost it quickly; in the VeriFish experience, anything beyond roughly three minutes was already too long for broad audience engagement. Videos also performed better when they included a personal or human dimension, while generic explanatory videos were easier to ignore. These findings suggest that communicating fisheries and aquaculture science effectively requires more than simplification. It requires

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translation into formats that fit different attention spans, expectations, and use contexts. Producers can act as valuable sources of information, especially for Tier 2 data, but communication products aimed at citizens must be short, visual, and easy to interpret. More detailed evidence can then sit behind those products in apps, factsheets, or linked materials. VeriFish therefore suggests that effective science communication in aquaculture depends on three conditions: a transparent and verifiable evidence base, audience-sensitive design, and the discipline to prioritise clarity over volume.

Acknowledgment

VeriFish has received funding from the European Union's Horizon Europe programme under Grant Agreement No. 101156426. The project focused on stimulating sustainable seafood consumption through a verifiable indicator framework.

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A CRITICAL EVALUATION OF CURRENTLY AVAILABLE WELFARE ASSESSMENT SYSTEMS FOR FARMED FISH: THE EURCAW-AQUA REVIEW

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Introduction

Fish welfare has become a central component of sustainable aquaculture, driving the development of welfare assessment schemes for a wide range of cultured species. However, species-specific differences in biology and welfare needs, together with variation in production systems and welfare definitions, have resulted in diverse assessments methodologies. This review aimed to critically examine currently available welfare assessment systems for farmed fish to identify common principles, strengths, and limitations that may inform the development of future protocols.

Materials and Methods

A comprehensive literature review of published welfare assessment systems and welfare guides developed for major aquaculture species, including Atlantic salmon (*Salmo salar*), European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*) and lumpfish (*Cyclopterus lumpus*), which is used as a cleaner fish in salmon aquaculture, was conducted. Assessment schemes were included in the review when they provided validated welfare indicators together with a classification or scoring system. Information was extracted regarding indicator selection, scoring and weighting methodologies, synthesis procedures, and the use of environmental-, management-, behavioral-, morphological-, and laboratory-based operational welfare indicators. Existing certification schemes incorporating welfare criteria were also examined. The full methodology is described in Samaras (2025).

Results and Discussion

The review identified substantial variation among welfare assessment systems regarding methods for indicator selection, weighting procedures, and synthesis to an overall welfare score. Holistic approaches, such as the Salmon Welfare Index Model (SWIM), combine environmental, management, and animal-based indicators into a single welfare score through semantic modelling (Stien et al., 2013). Similar holistic approaches have subsequently been adapted for other species, like rainbow trout (Tschirren et al., 2021) and European sea bass (Pavlidis et al., 2024). By contrast, some systems rely exclusively on external morphological indicators due to their welfare relevance, practicality and ease of implementation (Imsland et al., 2021; Weirup et al., 2022).

Although welfare indicators are generally evaluated for validity, many protocols rely partly on expert judgement to assign indicator scores, relative weights and synthesis algorithms, when used. This introduces an element of subjectivity and may limit comparability. Furthermore, many recently developed systems provide scoring frameworks for individual indicators but lack a validated method for generating an overall welfare score. Across species, recent studies increasingly emphasize practicality, on-farm applicability and stakeholder participation in decision-making through participatory approaches.

Conclusions

Fish welfare assessment has progressed rapidly in recent years, yet considerable variation remains among existing schemes. Unlike terrestrial livestock, farmed fish welfare currently lacks a standardized assessment framework, limiting comparability across species and production systems. Future welfare assessment schemes should be species-, production phase- and production system-specific, use scientifically validated and practical indicators, and include robust methods for weighting, aggregating, and interpreting welfare scores. Key areas for future development include positive welfare indicators, risk-based approaches, stakeholder involvement, regular updates reflecting emerging scientific evidence and independent verification. Addressing these priorities would improve reliability, practicality and broader adoption of fish welfare assessment.

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Acknowledgment

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EVALUATION OF ENVIRONMENTAL AND PHYSIOLOGICAL WELFARE INDICATORS DURING EXPERIMENTAL LIVE TRANSPORT OF JUVENILE RED SEABREAM (*Pagrus major*)

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Introduction

Live transport of fish is a routine but welfare-critical management procedure in aquaculture. Handling, crowding, confinement, deteriorating water quality, and exposure to novel environment can elicit acute physiological stress, the magnitude of which depends on transport duration, stocking density and the maintenance of suitable water quality. Species-specific information remains limited for emerging Mediterranean aquaculture species such as red seabream (*Pagrus major*). This study therefore evaluated environmental and physiological welfare indicators during short-duration transport under controlled conditions, with particular emphasis on liver cortisol as an indicator of stress and recovery.

Materials and Methods

Juvenile red seabream ($n = 300$; mean body weight = 2.63 g) were fasted for 24 h and transported from H.C.M.R. Crete to the University of Crete. Following water-level reduction and capture, fish were allocated to three 15-L transport tanks (~ 100 fish per tank; 17.5 kg m^{-3}). Transport lasted 30 min without water exchange, followed by complete water renewal upon arrival. Temperature, dissolved oxygen, pH, ammonia, nitrite, and nitrate were monitored throughout the procedure. Fish were sampled before water-level reduction (baseline), during loading, upon arrival, and 1, 2, and 5 days after transport. Liver cortisol was extracted following de Jesus et al. (1991) and quantified using a commercial ELISA kit (Neogen, UK) previously evaluated for red seabream. Preliminary validation supported liver cortisol as a proxy for whole-body cortisol.

Results and Discussion

The procedure comprised a 30-min preparation period (water-level reduction and fish capture) followed by 30 min of road transport, during which stocking density temporarily increased from 0.7 at the hatchery to 17.5 kg m^{-3} during transport, followed by a return to the initial density upon arrival. Water quality remained overall stable, wherein temperature and pH varied only slightly, dissolved oxygen declined moderately but remained within acceptable limits for the species, and no ammonia accumulation was detected.

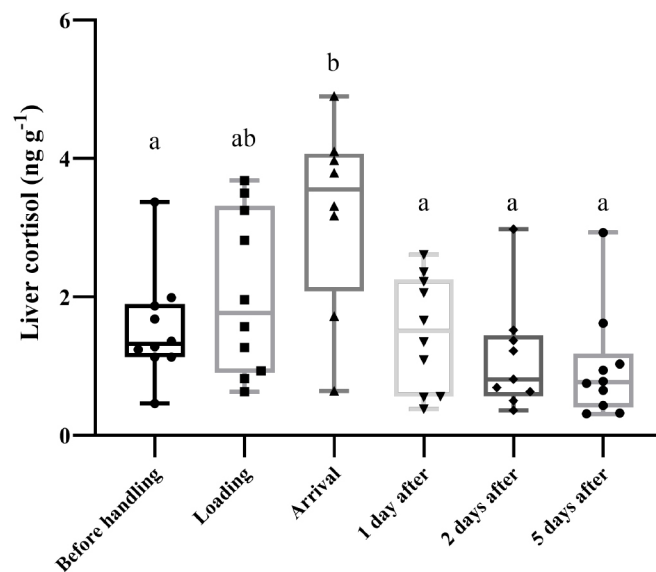


Figure 1. Liver cortisol concentration (ng g^{-1}) in red seabream at different stages of the transportation procedure ($n = 8\text{-}10$ individuals per experimental group). Different letters indicate statistically significant differences between groups.

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Transport stage significantly affected liver cortisol concentrations ($F_{5,51} = 5.973$; $p < 0.001$) (Figure 1). Concentrations were highest upon arrival and exceeded those at baseline and throughout recovery but did not differ significantly from values recorded during loading, as has been previously reported in larger fish of the species (Samaras et al., 2022). Cortisol returned to baseline levels by day 1 and remained low on days 2 and 5. This rapid recovery indicates that the physiological response was acute and reversible. Because water quality remained suitable, the response was more likely associated with handling, loading, temporary crowding, and confinement than with environmental deterioration. Thus, short-duration transport under well-controlled conditions appears to represent a manageable welfare challenge for juvenile red seabream, provided that loading procedures, stocking density, and water quality are carefully managed.

Acknowledgment

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THE MINERALOCORTICOID RECEPTOR MODULATES RESPONSES TO EARLY-LIFE CORTISOL EXPOSURE IN ZEBRAFISH

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Introduction

Early-life stress can induce long-lasting effects on physiology, growth, and behavior through activation of the hypothalamic–pituitary–interrenal (HPI) axis and increased cortisol secretion. Cortisol exerts its actions primarily through the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR), which play complementary roles in stress regulation. While GR-mediated mechanisms have been extensively investigated, the role of MR in shaping responses to elevated cortisol during early life remains incompletely understood. This study investigated whether the timing and duration of exogenous cortisol exposure during early development influenced larval growth, trunk cortisol levels, and locomotor behavior in wild-type (WT) and MR knockout (MR^{-/-}) zebrafish, thereby providing mechanistic insight into early life stress responses in teleosts.

Materials and Methods

Adult WT (Tübingen line) and MR^{-/-} zebrafish (Dinarello et al., 2022) were maintained at the Zebrafish Facility, Fish Physiology Laboratory, University of Crete, under standard conditions (28°C; 14 h light:10 h dark photoperiod) in a recirculating system. To ensure synchronized fertilization, breeders were manually stripped, and fertilized embryos from individual breeding pairs were collected. At 30 min post-fertilization, embryos were allocated to 6-well plates (20 embryos/well) containing either egg water (controls) or egg water supplemented with cortisol (water-soluble hydrocortisone, 1 µg/mL; Sigma-Aldrich). Embryos remained under the assigned treatment until hatching, after which larvae were maintained until 5 days post-fertilization (dpf) in either fresh control medium or fresh medium supplemented with cortisol. This experimental design generated four treatment groups: (1) Control (no cortisol exposure), (2) Early Cortisol (cortisol exposure until hatching only), (3) Late Cortisol (cortisol exposure from hatching to 5 dpf), and (4) Chronic Cortisol (continuous cortisol exposure from fertilization to 5 dpf). At 5 dpf all fish were transferred to clean medium, to allow for a one-day wash-out period, and at 6 dpf larvae were assessed for growth, basal whole-trunk cortisol concentrations and behavior responses during the Light–Dark Transition Test (LDTT) using the DanioVision tracking system. Cortisol was quantified in individual larvae, following the protocol of Samaras & Pavlidis (2020), using a validated ELISA assay. Locomotor behavior was quantified with EthoVision XT v15. Statistical analysis was performed using linear mixed-effects models in RStudio.

Results and Discussion

At 5 dpf, control larvae had shorter body length than larvae in the Early ($p < 0.01$) and Late Cortisol ($p = 0.015$) treated fish across both genotypes. By 6 dpf, these differences were no longer evident. Preliminary analyses further indicated that cortisol exposure may affect whole-body cortisol concentration in larvae. Locomotor activity in the LDTT test was influenced by the Genotype x Treatment ($p < 0.001$), and Genotype x Light-Dark period interactions ($p = 0.016$) (Fig. 1). Post-hoc analyses confirmed that larvae of both genotypes were more active during the dark than during the light phases, consistent with the expected behavioral response (Faught & Vijayan, 2018). Across all treatments, MR^{-/-} larvae displayed higher activity than WT larvae, except in the Late and Chronic Cortisol exposed fish during the dark phases. While cortisol treatment did not affect locomotor activity in WT fish, cortisol exposed MR^{-/-} groups exhibited significantly reduced activity compared with untreated MR^{-/-} controls. These findings suggest that functional MR signaling buffers the behavioral effects of cortisol exposure, allowing WT larvae to maintain normal locomotor responses despite elevated cortisol. In contrast, the lack of MR resulted in hyperactivity under basal conditions, while cortisol exposure attenuated this phenotype, potentially through GR-mediated signaling.

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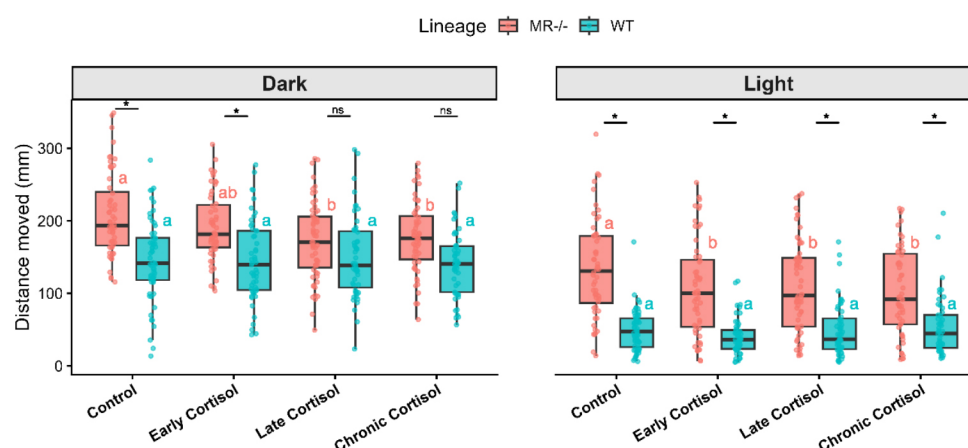


Fig. 1. Mean activity (distance moved, mm) during dark and light phases in 6 dpf WT and MR^{-/-} zebrafish larvae exposed or not (Control) to exogenous cortisol during different developmental stages (n = 51-60 per treatment, N = 3 independent experiments). Different letters indicate statistically significant differences among treatments within each genotype, and asterisks (*) indicate statistically significant differences between genotypes within each treatment.

Funding

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ASSESSMENT OF JUVENILE EUROPEAN SEABASS (*Dicentrarchus labrax*) WELFARE DURING IMMERSION AND INJECTABLE VACCINATION

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Introduction

Vaccination in aquaculture is an essential preventive strategy for reducing disease occurrence and limiting pathogen prevalence, thereby contributing to fish health and welfare. Despite its benefits, the vaccination process itself can act as a stressor and may pose welfare risks, depending on the vaccination method and handling procedures applied. Although producers increasingly aim to implement practices that minimize stress and improve welfare outcomes, the absence of a widely accepted handbook of good vaccination practices in Mediterranean aquaculture has resulted in the development of company-specific protocols and operating procedures. This study evaluated the stress responses and suitable welfare indicators associated with vaccination procedures in European seabass (*Dicentrarchus labrax*) under commercial aquaculture conditions. Two commonly applied vaccination strategies were investigated, namely immersion vaccination combined with size grading in land-based pre-on-growing tanks and injection vaccination performed in sea cages.

Materials and Methods

Vaccination practices used in commercial Mediterranean aquaculture were first documented and mapped across companies and production stages to define operational workflows and sampling points. Water quality (temperature, dissolved oxygen and pH) and biological samples were collected during the two commercial procedures. The stress response was assessed through tissue cortisol analysis. Specifically, whole-body samples were collected from small fish during the pre-on growing vaccination, whereas liver samples were collected from fish undergoing vaccination in sea cages. A preliminary validation confirmed liver cortisol as a suitable proxy for trunk cortisol ($r = 0.655$, $n = 20$, $p = 0.002$) in small fish. Cortisol was extracted according de Jesus et al. (1991) and quantified using a commercial ELISA kit (DRG, Germany) previously evaluated for European seabass samples.

Results and Discussion

Under commercial pre-on-growing conditions, immersion vaccination combined with size grading induced a measurable but transient stress response in European seabass, as handling is known to elevate cortisol levels in this species (Fanouraki et al., 2011). Cortisol concentrations differed significantly among sampling stages ($F_{8,66} = 17.59$, $p < 0.001$), with levels progressively increasing throughout the handling procedure and reaching statistically significant elevations after vaccination and grading were completed (Figure 1a). Despite the prolonged crowding period (1 h 27 min), environmental conditions remained stable, with adequate oxygenation maintained throughout handling. Notably, cortisol concentrations returned to baseline values within 24 h post-handling and decreased further after 48 h (Figure 1a), indicating rapid physiological recovery and suggesting that, when under the commercial conditions evaluated, immersion vaccination and grading represent an acute but reversible welfare challenge.

Injection vaccination performed in sea cages resulted in a significant increase in liver cortisol concentrations immediately after handling in both evaluated batches ($F_{4,43} = 6.678$, $p < 0.001$), while values recorded 24 h later showed partial recovery toward pre-vaccination levels. The procedure involved repeated handling cycles and approximately 3 min of cumulative handling per fish, yet environmental parameters remained within normal ranges. Mortality showed a peak two days after vaccination and gradually declined thereafter, reaching a cumulative value of 0.8% by day 6.

Overall, both vaccination approaches elicited short-term stress responses, but the observed recovery patterns and low mortality indicate that current commercial practices can maintain acceptable welfare outcomes when appropriate handling and environmental management measures are applied.

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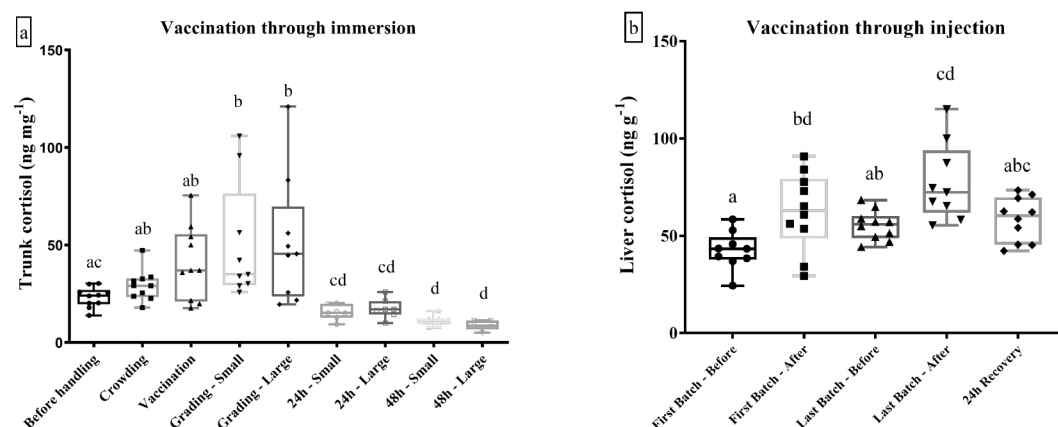


Figure 1. (a) Trunk cortisol concentration (ng g^{-1}) in European seabass at different stages of the vaccination and grading procedure ($n = 6\text{--}10$ individuals per experimental group). (b) Liver cortisol concentration (ng g^{-1}) in European seabass at different stages of the vaccination procedure ($n = 10$ individuals per experimental group). Different letters indicate statistically significant differences between groups.

Acknowledgment

This research was funded by the European Union – NextGenerationEU through the National Recovery and Resilience Plan Greece 2.0, under the project “Development of innovative tools and services for the assessment of aquaculture fish welfare (CARFISH-TRN)” (MIS 5180519), within the framework of the action Clusters of Research Excellence (CREs). The authors would like to thank the companies, which are not identified here to preserve confidentiality, that generously provided the biological material used in this study.

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GENETIC AND ENVIRONMENTAL EFFECTS ON EPIGENETIC VARIATION ASSOCIATED WITH THERMAL PLASTICITY AND BODY WEIGHT IN EUROPEAN SEABASS POPULATIONS

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Introduction

Environmental temperature is a critical factor influencing the physiology and growth of marine teleosts. Epigenetic mechanisms, particularly DNA methylation, are increasingly recognized as potential mediators of phenotypic plasticity and short-term adaptation to climate change. However, the interplay between these epigenetic responses and the underlying genetic background remains poorly understood. In this study, we investigated the relationship between temperature, population origin, and the liver methylome in the European sea bass (*Dicentrarchus labrax*), a species with important ecological and economic value (Vandeputte et al., 2019) although modern rearing techniques date back only from the late 1980s. It also has high interest for evolutionary studies, as it is composed of two semispecies (Atlantic and Mediterranean lineages). While previous work identified significant population differentiation in the methylome of the liver across populations ($P = 0.004$) linked to genome architecture (Sánchez-Baizán et al., Submitted) may significantly influence fish performance and short-term adaptation. However, their relationship with gene expression (GE), those results were limited to a single thermal regime. The current study expands this by examining how different populations respond to three thermal environments at the whole-methylome level while accounting for genetic variation.

Materials and methods

We implemented a robust experimental design involving ~4,000 individuals of from two distinct populations (Atlantic, AT; and East Mediterranean, EM). Fish were exposed to three different temperature regimes (mean temperatures: 14.5, 17 and 21.8°C) during two years (Crestel et al., 2025). At the end of the trial, liver samples were collected from 180 individuals. Phenotypic data, including body weight, length, Hepatosomatic Index (HSI), and condition factor (K), were recorded. We used whole-genome enzymatic methyl-seq (EM-seq) to analyze DNA methylation levels at single-nucleotide resolution. Bioinformatic analysis was performed using Methyl-seq nextflow pipeline (Ewels et al., 2025). Furthermore, we extracted SNPs from the same reads using BISCUIT package (Zhou et al., 2024). We performed variance partitioning through Redundancy Analysis and conducted meQTL mapping to differentiate between genetically determined and autonomous epigenetic marks. This will be complemented by applying regression-based models to identify DNA methylation levels associated with phenotypic traits of interest (e.g., Methylation ~ Population x Temperature x Body weight) while controlling for multiple testing.

Results and Discussion

The EM-seq sequencing effort produced a total of 926 Gb of high-quality data, with a mean alignment rate of 82% across the 180 libraries. Preliminary statistical analyses revealed significant differences in the methylome between the AT and EM populations, as well as a response to temperature treatments. Our ongoing analysis utilizes variance partitioning and meQTL mapping to dissect the source of this variation, specifically distinguishing between genetically- and environmentally-induced epigenetic marks. This distinction is critical to quantify the autonomous potential of the sea bass epigenome to contribute to evolution and thermal plasticity (Mueller et al., 2025). By categorizing identified regions into pure (environment), facilitated (genetic x environment), and obligated (genetic) epialleles, we aim to determine the extent to which these marks provide a substrate for selection. Furthermore, associating the direction of methylation change with continuous traits like body weight and HSI allows us to identify the regulatory mechanisms underpinning thermal short-term adaptation and robustness in different genetic backgrounds.

Conclusions

This study disentangles genetic and environmental contributions to methylation variation, revealing the methylome as a dynamic regulatory layer underlying population-specific thermal plasticity. Ongoing quantitative analyses will refine the relative roles of genetic control and autonomous epigenetic variation, providing insights into plasticity with direct relevance for aquaculture and ecological robustness under climate change.

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Table 1. Experimental design matrix (N=180), ensuring 30 biological replicates per group, providing sufficient statistical power for quantitative epigenetic modeling—a sample size that significantly exceeds standard practice in high-resolution whole genome studies.

Population	14.5°C	17°C	21.8°C	Total
AT	30	30	30	90
EM	30	30	30	90
				180

Acknowledgements

This study is supported by the ANR FishNess project, CRECHE project (P120-0001-01-DF), and an Ifremer postdoctoral fellowship (2023–2026) awarded to NSB.

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ARTIFICIAL REEFS UNDER MUSSEL AQUACULTURE RAFTS AS HABITAT FOR COMMERCIAL INVERTEBRATES: SPATIOTEMPORAL DYNAMICS OF INVERTEBRATE COMMUNITIES AND POST-RELEASE BEHAVIOUR OF EUROPEAN LOBSTER (*Homarus gammarus*)

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Introduction

Suspended mussel aquaculture systems represent a dominant feature of productive coastal areas in NW Iberian Peninsula, substantially modifying benthic environments through shell deposition, organic enrichment, and the addition of secondary hard substrate. While these modifications can increase habitat heterogeneity and attract mobile benthic fauna, their potential for restocking species of commercial interest remains poorly quantified (Carbonell-Garzon et al., 2026). The objectives of this study were to evaluate the capacity of artificial reef (AR) structures deployed beneath mussel rafts and on adjacent sandy substrates to enhance invertebrates species of ecological and commercial importance; to determine whether habitats associated with suspended mussel aquaculture can retain deliberately released individuals of European lobster *Homarus gammarus* as a potential stock enhancement strategy; to assess whether the origin of released lobsters (aquaculture-reared versus wild-translocated) influences their post-release spatial behaviour and site fidelity.

Material and Methods

Study site and AR deployment: The study was conducted from October 2023 to September 2024 in the central mussel raft polygon of the outer Ría de Vigo (NW Spain; 42°14'49" N, 008°49'15" W; ~0.36 km²; 35 mussel raft). In May 2023, twelve concrete AR modules were installed at 14–16 m depth across four sites: two beneath mussel rafts (AR-M) and two on adjacent sandy substrates (AR-S), each comprising three blocks with removable sampling plates for non-destructive monitoring. An open sandy habitat (Sand) served as an unstructured control. Monthly diver-based visual censuses were carried out, estimating relevant invertebrates abundance, over the full 24-month period. Occurrence frequencies of commercially important taxa were recorded at AR-M and AR-S sites. Moreover, we used acoustic telemetry tools to study the post-release spatial behaviour and site fidelity of of a 46 *H. gammarus* (24 aquaculture-reared, 22 wild-translocated) released across three habitat configurations (AR-M, AR-S, and mussel-raft mooring without reef, MR). All individuals were tagged with Thelma Biotel LP-6 transmitters in two release periods (October 2023 and April 2024) and released. An array of 20 Thelma Biotel receivers (TBR-700L/800) monitored the central and peripheral polygons of mussel rafts in the study area, including the artificial reefs. Data were obtained in September 2024.

Results

Artificial reefs under mussel rafts (AR-M) generated significantly higher occurrence frequencies of commercially important species: *Paracentrotus lividus* (72.2% vs. 11.1%), *Octopus vulgaris* (25.9% vs. 16.7%), and *Necora puber* (20.4% vs. 0.93%) compare to AR-S. Differences were only significant during spring and summer months. During the first 72h after release, 27.9% of the 43 released lobsters remained close to the release site, 48.8% redistributed within the acoustic array, and 23.3% emigrated out site of the monitored area. This behavior differed significantly between origin groups (Fisher's exact test, $p = 0.037$), with 55.6% of aquaculture-reared individuals remained at the release receiver during hours de first 72h, compared with only 13.3% of wild-translocated individuals. Long-term residency and space-use metrics (Kernel density) differed by origin; wild individuals tended towards larger space use and earlier departure from the central array.

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Discussion

The results confirm that AR deployment beneath suspended mussel rafts creates structurally and functionally complex microhabitats capable of supporting diverse benthic communities and retaining species of ecological and commercial value. The pronounced enhancement of species abundance at AR-M compared with AR-S suggests that the combined effect of hard substrate and aquaculture-derived organic enrichment — including shell accumulation and particulate organic matter fallout — acts synergistically to increase habitat suitability. The elevated occurrence of *P. lividus*, *N. puber*, and *O. vulgaris*, at mussel-raft-influenced AR sites is consistent with previous observations of aquaculture infrastructure functioning as de facto artificial habitat for mobile fauna. The behavioural divergence between aquaculture-reared and wild-translocated lobsters has direct implications for restoration program design. Higher site fidelity among hatchery-reared individuals during the critical post-release window may reflect reduced exploratory behaviour associated with captive rearing, rather than active habitat preference; longer monitoring is required to determine whether this translates into sustained retention or eventual dispersal. Wild-translocated individuals, despite showing broader initial movement across the array, may draw on prior experience of natural environments to locate and establish shelter in unfamiliar release areas. Together, these findings support a differentiated release strategy tailored to individual origin and habitat configuration. This work demonstrates the feasibility of integrating ecological restoration objectives into working aquaculture seascapes through targeted artificial reef deployment under mussel farms. The results provide a replicable model for ecological mitigation in aquaculture-modified coastal environments, directly relevant to EU Nature Restoration Law implementation in areas where productive use and biodiversity recovery must be reconciled.

Acknowledgment

This study was funded by the EU Horizon Europe project CLIMAREST: Coastal Climate Resilience and Marine Restoration Tools for the Arctic Atlantic basin (grant agreement 101093865).

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CLIMATE-DRIVEN CHANGES IN THE MEDITERRANEAN SEA ESTIMATED THROUGH THE THERMAL ANOMALY STRATIFICATION INDEX (TASI): IMPLICATIONS FOR MARINE AQUACULTURE

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Introduction

Climate change is intensifying sea surface temperatures across the globe, with particularly pronounced effects in the Mediterranean Sea, a semi-enclosed basin where marine heatwaves have become more frequent and intense. While most climate risk assessments for aquaculture rely on sea surface temperature (SST) alone, this approach fails to capture sub-surface thermal dynamics that directly affect farmed fish confined within cage structures. This study analyses changes in vertical thermal stratification at 12 Mediterranean aquaculture facilities across seven countries over a 35-year period (1987–2021), providing a water-column perspective on climate risk for the sector.

Material and Methods

Three-dimensional monthly temperature data (0–50 m depth) were retrieved from the Copernicus Marine Service reanalysis product (MEDSEA_MULTIYEAR_PHY_006_004) and averaged over $\sim 14 \times 14$ km grids around each facility in Spain, Croatia, Tunisia, Greece, Türkiye, Cyprus and Egypt. Data were divided into a reference period (1987–2010) and a post-period (2011–2021). Thermal Anomaly Stratification Index (TASI), a normalised Root Mean Square Error Index, measuring deviation of isotherm areas (20–28°C) from the historical baseline, was calculated for each year.

Results and Discussion

TASI values followed a clear longitudinal and latitudinal gradient, with the highest mean values in South Türkiye (0.713), North Türkiye (0.564) and Tunisia (0.524), and the lowest in East Spain (0.222). At two Turkish stations, TASI exceeded 1.0 in individual years, indicating that stratification anomalies surpassed the historical thermal signal itself. These patterns have direct consequences for gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*), the dominant farmed species in the region, whose optimal thermal range (18–24°C) is progressively compromised by the deepening of warm water masses within cage depth ranges. Based on the observed TASI distribution, a provisional risk classification framework is proposed (low: TASI < 0.3; moderate: 0.3–0.7; high: > 0.7) to guide adaptive management decisions including adjustments to feeding protocols, stocking densities and cage relocation (Figure 1).

Conclusions

Vertical thermal stratification in the Mediterranean Sea has intensified significantly since 2011, with the strongest changes in the eastern and southern basin. TASI constitutes a robust, transferable tool for translating climate data into actionable risk levels for aquaculture managers and spatial planners. Incorporating water-column thermal dynamics into Allocated Zones for Aquaculture (AZA) selection and farm-level management is essential to ensure the long-term sustainability of Mediterranean marine aquaculture under accelerating climate change.

Acknowledgments

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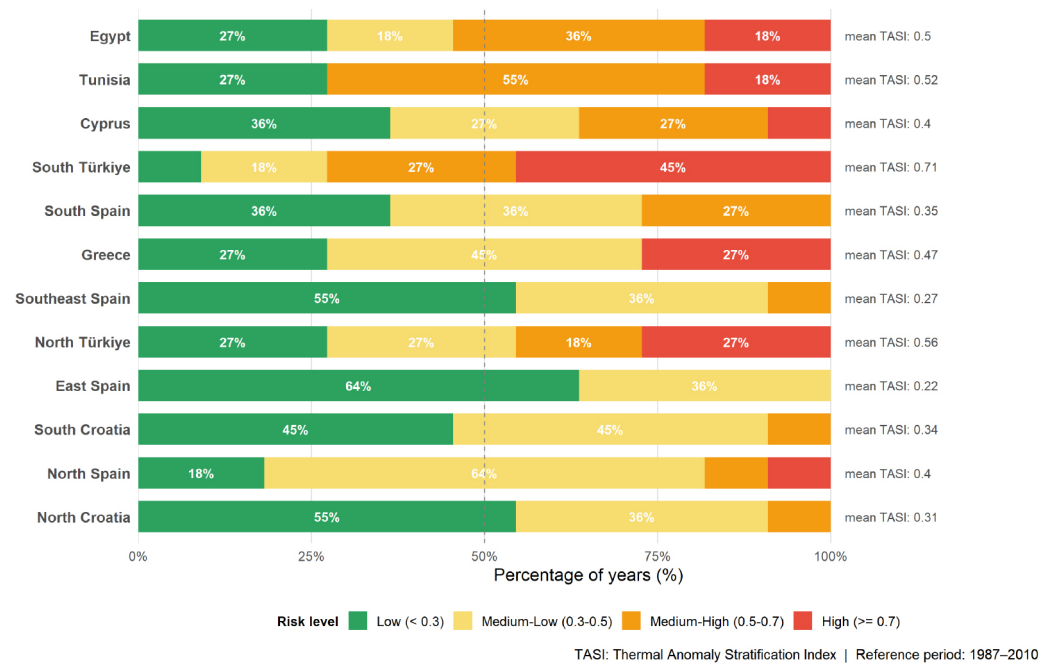


Figure 1. Probability of the vertical thermal stratifications risk across different AZAs in the Mediterranean Sea using TASI (Thermal Anomaly Stratification Index), from 2011 to 2021, with the period 1987-2010 as the reference baseline.

FEEDING SALMON PROTEIN HYDROLYSATE TO SALMON: A SAFE AND CIRCULAR SOLUTION FOR SUSTAINABLE AQUACULTURE

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Introduction and methodology

Utilization of fish by-products as feed ingredients may be key to improving sustainability in aquaculture of Atlantic salmon (*Salmo salar*). Salmon protein hydrolysate, produced by enzymatic hydrolysis of salmon by-products, represents a circular feed ingredient with favourable nutritional characteristics. This study represents one of the first long-term commercial-scale demonstrations of salmon protein hydrolysate inclusion throughout the marine production phase. Atlantic salmon (*Salmo salar*) were fed either a standard commercial control diet or an experimental diet in which part of the plant protein fraction was replaced with 5 % salmon protein hydrolysate. Salmon post-smolts (initial mean body weight approximately 120 g), originating from the same hatchery, were distributed into four sea pens (two replicates per diet) and followed for approximately 16 months until near slaughter size (final mean body weight approximately 5 kg). Sampling of fish was performed 2 weeks prior to final weighing of all fish and 2.5 months prior to slaughter, which was staggered over several dates per pen.

Results and discussion

Growth performance, biological feed conversion ratio, slaughter quality, and fillet proximate composition were comparable between dietary groups. Health-related parameters, including mortality, histological evaluations of liver and intestinal tissues, and blood plasma biochemistry, indicated no signs of compromised fish health associated with long-term feeding of salmon protein hydrolysate. Plasma osmolality was significantly higher in salmon fed the hydrolysate-containing diet, suggesting a potential influence on osmoregulatory processes. Overall, the results support partial replacement of plant protein with 5 % salmon protein hydrolysate as a circular and sustainable feeding strategy for Atlantic salmon farming.

EVALUATION OF METABOLIC RESPONSES OF COMMON CARP (*Cyprinus carpio*) JUVENILES TO DIETS DIFFERING IN DIGESTIBLE ENERGY AND PROTEIN RATIO FED AT LOW WATER TEMPERATURE

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Introduction

Increasing water temperatures during winter can significantly affect pond aquaculture, as fish that would normally overwinter near the pond bottom in a fasting state become more active and exhibit increased feeding behavior. However, despite these warmer conditions, the natural plankton supply remains limited. Consequently, farmers are increasingly interested in feeding fish stocks during warming winter periods, although the optimal diet composition and its effects on nutrient utilization under these conditions remain poorly understood. Within the framework of the ACTFAST project, we aimed to formulate a functional off-season feed for common carp and to develop feeding protocols that mitigate the combined effects of winter stressors while enhancing growth performance. In this paper, we present the first results on feed formulation and its nutritional effects in juvenile common carp reared at low water temperature. Growth performance data are presented separately by Terhes et al.

Materials and Methods

Four diets for carp yearlings were formulated and produced by Garant Ltd. (Austria). The diets differed in their digestible protein to digestible energy ratios (DP/DE, g/MJ): Diet 1: 18.6; Diet 2: 15.4; Diet 3: 13.8; and Diet 4: 11.4. Winter wheat was included as a negative control diet (Diet 5.), as it is still commonly used by farmers. Diet 1 was formulated to have a DP/DE ratio similar to that of a standard on-growing diet. The diets were formulated to have high /low protein and high /low fat content, respectively. The feeding trial lasted 55 days and was conducted in a recirculating aquaculture system (RAS) with three replicate tanks per treatment. The initial body weight of the fish was 99.5 ± 13.8 g, and the stocking density was 35 fish/m³. The feeding rate was set at 1% of biomass according to Terhes et al., 2025. At the end of the trial, 3-3 fish/tank were sampled for determination of biochemical parameters and enzyme activities in the blood and liver. The blood samples for biochemistry were collected from the caudal vein of fish using a heparinized syringe and tubes. The heparinized tubes were then centrifuged with 1700 rcf for 20 min at 4 °C to obtain plasma and stored at -20°C until analysis. Additionally, the liver was dissected, immediately frozen in liquid nitrogen and stored at -80 °C for analysis of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzyme activities. The trial then continued with the remaining fish at 20 °C, where the stocking density was 19 fish/m³ (approximately 2.2 kg/m³). All groups were fed with Diet 1 at a feeding rate of 3% of biomass. At the end of this 43-day follow-up period, blood biochemical parameters were again assessed.

Results and Discussion

Dietary treatments significantly affected several biochemical indices, while others remained unchanged. Cholesterol were significantly ($p = 0.036$) higher in dietary group 4 (372.9 ± 85.2 mgdL⁻¹) while total bilirubin ($p = 0.007$) in group 5 was higher. Total protein (TP) ($p = 0.019$), albumin ($p < 0.001$), and phosphorus (P) ($p = 0.019$) were significantly affected, with the lowest values typically observed in the wheat-based control group. The blood urea nitrogen-to-creatinine ratio (B/C) was also significantly different among treatments (values in range 7.7-22.4). In contrast, lipase, ALT, ALP, calcium, creatinine, glucose, globulin, and total antioxidant capacity showed no significant differences among dietary groups ($p > 0.05$), although some numerical variation was observed. Overall, the results indicate that dietary protein and fat levels primarily influenced protein metabolism-related indicators (e.g., TP, albumin, B/C) and certain liver-associated biochemical markers, while most enzymatic and metabolic parameters remained stable across treatments. When comparing the biochemical parameters of fish blood plasma reared at 10°C and 20°C, TP, creatinine, glucose level, Ca and P content, and activity of ALT enzyme were differing significantly ($p < 0.05$). This observation suggest that water temperature exerted a stronger regulatory effect on fish physiology than dietary protein and lipid levels.

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Hepatic ALT activity ranged from 5.35 to 7.60 mmol/mg protein and did not differ significantly among the dietary groups ($p = 0.054$), although the lowest activity was recorded in Diet 5. Likewise, AST activity showed no significant dietary effect due to high individual variation, with values ranging from 1.39 to 2.85 mmol/mg protein. The higher ALT activity observed in the liver of fish fed the low-protein diet may indicate an increased endogenous amino acid turnover or enhanced protein catabolism to compensate for the reduced dietary protein supply. However, this finding should be interpreted with caution, as ALT is not only a marker of hepatic damage but also a key enzyme involved in amino acid metabolism and transamination reactions. Therefore, the elevated ALT activity may reflect metabolic adaptation to altered nutrient availability rather than impaired liver function.

Conclusions

Overall, the results suggest that juvenile carp maintained a relatively stable biochemical status despite considerable differences in dietary protein and lipid levels, indicating a high degree of metabolic adaptability during winter feeding. Dietary protein level exerted a more pronounced effect than dietary lipid level, mainly influencing indicators related to protein metabolism and liver function, whereas water temperature emerged as the dominant factor regulating biochemical responses.

Acknowledgements

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ASSESSING THE VALUE OF SINGLE CELL PROTEINS IN SALMONID PRODUCTION

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Objective and design

This study evaluated the use of a novel single cell protein (SCP, Protopia) as a sustainable alternative to fish meal and soy protein concentrate in rainbow trout (*Oncorhynchus mykiss*) diets. The feeding trial was conducted at CRNA (Village-Neuf, France) over 11 weeks, using juvenile trout with an initial body weight of 90.65±0.46 g. Five dietary treatments were formulated: a commercial-like control and four diets with increasing SCP inclusion levels (2.5%, 5%, 10%, and 15%), each in triplicate. Feed formulations were balanced for crude protein (~38.5%) and lipid (~24.4%), with amino acid supplementation to ensure nutritional adequacy. Growth performance, digestibility and immune response were assessed after 77 days of experimental feeding.

Results and conclusions

Results indicate that all SCP inclusion levels support comparable body weight gain and final body weight to the control diet. Specifically, final body weight ranged from 342.2g to 350.0g, across treatments. Results show that the tested SCP can replace conventional protein sources in salmonid diets while maintaining growth performance and improving protein (Figure 1) and selected amino acid digestibility (Figure 2) at higher inclusion levels, without compromising immune function or nutrient retention.

In summary, the trial demonstrates that SCP can replace fish meal and soy protein concentrate up to 15% without compromising growth or health under experimental conditions.

These findings support SCP as a promising, sustainable protein source for aquafeeds, contributing to responsible consumption, and life below water (SDGs 2, 12, 14). These results indicate that value creation from SCPs lies not only in sustainable protein supply, but in predictable nutrient utilization, supporting precision feed formulation. Targeted inclusion levels (10–15%) should be prioritized in future development to maximize digestibility benefits while safeguarding gastrointestinal functionality.

Figures

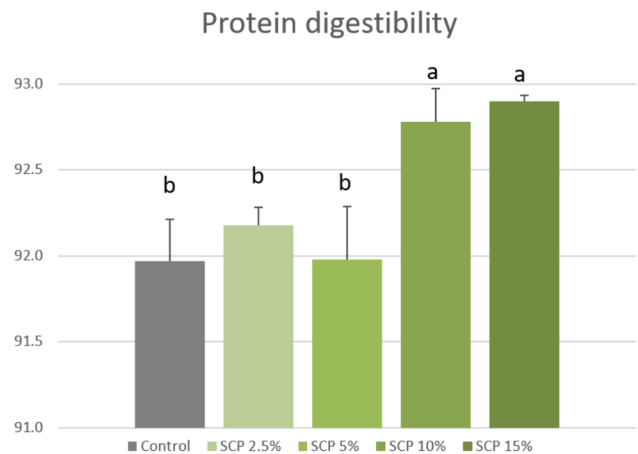


Figure 1: Protein apparent digestibility coefficient (%). Data are presented as mean ± SD (Standard Deviation). Significant differences (p<0.05) are shown by different letters. N=3.

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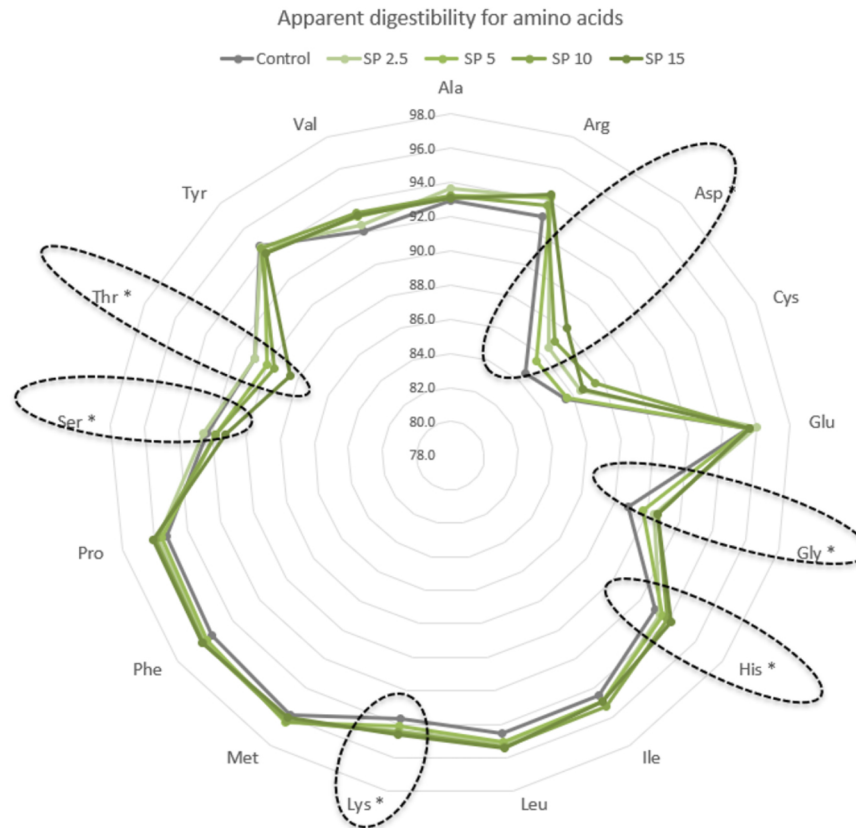


Figure 2: Apparent digestibility for individual amino acids. Significant differences ($p < 0.05$) are shown by an asterisk. $N=3$.

AZA4ICE: A MULTI-CRITERIA DECISION-SUPPORT FRAMEWORK FOR CIRCULAR ALLOCATED ZONES FOR AQUACULTURE (C-AZA) DEVELOPMENT AND VALIDATION ACROSS EUROPEAN PILOT SITES

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Introduction

The sustainable expansion of aquaculture requires spatial planning tools capable of integrating environmental sustainability, production efficiency, circular economy principles and socio-economic development. Although Allocated Zones for Aquaculture (AZAs) are widely recognised as an effective instrument for aquaculture planning, this approach is based primarily on the assessment of environmental suitability and site selection, but with limited integration of ecosystem functioning, circular production systems and stakeholder participation (Sánchez-Jerez et al., 2016; Macías et al., 2019). To address these gaps, the Interreg Euro-MED AZA4ICE project developed an ecosystem-based decision-support framework for identifying Circular Allocated Zones for Aquaculture (C-AZA). The methodology integrates environmental suitability assessment, prioritisation of candidate species, evaluation of Integrated Multi-Trophic Aquaculture (IMTA) system performance, circularity assessment and participatory governance into a harmonised workflow applicable across a wide range of aquatic ecosystems. This study presents the AZA4ICE methodology and its validation through coordinated implementation across 8 European pilot sites.

Materials and Methods

The AZA4ICE framework consisted of three sequential analytical modules based on a Multi-Criteria Decision Analysis (MCDA). The first module evaluated Site Suitability Index (SiSI) by integrating environmental variables, water quality, trophic status, ecosystem characteristics, infrastructure, competing water uses, risk factors and regulatory constraints. Variables were standardised using fuzzy membership functions and combined through weighted linear aggregation to generate site suitability scores. The second module assessed Species Suitability Index (SSI) by comparing local environmental conditions with the ecological requirements, physiological tolerance, technological feasibility, market potential and regulatory compatibility of potential farming species. Candidate species were evaluated according to their functional role within IMTA systems, including fed species, organic extractive species and inorganic extractive species. The third module evaluated circular aquaculture performance through the assessment of potential IMTA configurations based on trophic complementarity, nutrient recycling capacity, production feasibility and market value. These criteria were integrated into the IMTA Circularity Index (ICI) and combined with the previous assessments to calculate the final Circular Allocated Zone for Aquaculture Index (C-AZAi). Stakeholder engagement supported all analytical phases through data validation, interpretation of results and refinement of management scenarios.

Results

The methodology was developed, implemented and validated across eight pilot sites located in eight European countries, representing highly contrasting aquaculture environments, including coastal lagoons, estuarine systems, marine bays and freshwater ecosystems. The pilot testing network encompassed diverse ecological conditions, governance frameworks, aquaculture production systems and levels of data availability, providing a comprehensive evaluation of the framework under real operational conditions. The complete analytical workflow was successfully applied in all pilot sites, including Site Suitability Assessment, Species Suitability Assessment, IMTA evaluation, stakeholder validation and final C-AZA delineation, without requiring structural modifications to the methodological framework. Across the pilot network, a total of 24 candidate aquaculture species and 40 IMTA configurations were evaluated according to local environmental conditions and production systems. Despite substantial variability in ecosystem characteristics and monitoring intensity, the methodology generated harmonised and comparable outputs, with C-AZAi values ranging from 0.63 to 0.79, demonstrating high methodological robustness and transferability. Cross-case analysis further showed that circular aquaculture performance was primarily determined by functional trophic complementarity and coherent IMTA design, rather than by species richness or system complexity alone, highlighting the importance of ecosystem functioning in the identification of circular aquaculture solutions.

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Discussion

The AZA4ICE methodology extends conventional aquaculture zoning approaches by integrating environmental suitability, species performance, circular economy principles and stakeholder knowledge within a single ecosystem-based decision-support framework. Its successful validation across heterogeneous European pilot sites demonstrates that the methodology is both operationally robust and transferable while remaining sufficiently flexible to accommodate local environmental, technological and governance conditions. Beyond its application within the project, the methodology is supported by a suite of openly available scientific outputs, including methodological guidelines, pilot applications, comparative analyses and a geospatial platform, providing transparent and reproducible resources for researchers, planners and public authorities (Porporato et al., 2026). By supporting the identification of Circular Allocated Zones for Aquaculture through an ecosystem-based approach, the framework represents a practical tool for implementing Maritime Spatial Planning and advancing the transition towards a more sustainable, resilient and circular European aquaculture sector.

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A FLEXIBLE METHODOLOGY FOR IDENTIFYING ALLOCATED ZONES FOR DIVERSIFIED AQUACULTURE SYSTEMS

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Introduction

Spatial planning has become a fundamental component of sustainable aquaculture development, supporting the identification of suitable farming areas while balancing environmental protection, socio-economic needs, and competing uses of aquatic ecosystems. The increasing competition for coastal space has highlighted the need for effective planning approaches capable of reducing conflicts and promoting sustainable aquaculture development (Sanchez-Jerez et al., 2016). In this context, Allocated Zones for Aquaculture (AZA) have been developed as a strategic spatial planning tool to support the sustainable allocation of marine and coastal areas for aquaculture activities (Macias et al., 2019). Existing AZA methodologies have mainly focused on marine and coastal environments and have generally relied on a relatively consistent set of environmental, technical, and regulatory criteria. However, the diversification of aquaculture towards low-trophic species, organic farming, restorative aquaculture, Bio-Integrated Multi-Trophic Aquaculture (Bio-IMTA) and aquaponics introduces new planning challenges. These production systems differ substantially in their ecological processes, operational requirements, spatial constraints, and ecosystem services, requiring approaches that move beyond a single standardised suitability model.

Within the Horizon Europe DIVERSIFY4Future project, Work Package 8 is developing a harmonised yet flexible methodology for identifying AZA across marine, transitional and freshwater environments, adapting spatial planning approaches to the characteristics of diversified aquaculture systems while maintaining methodological consistency among European regions.

Methodology

The proposed methodology combines ecosystem-based spatial planning principles, GIS, environmental modelling, remote sensing, and participatory governance within a modular decision-support framework. The workflow begins with the harmonisation of spatial datasets describing aquaculture production, environmental conditions, ecosystem characteristics, protected areas, regulatory constraints, infrastructure, competing water uses and socio-economic drivers. These datasets provide a common geospatial foundation for planning across different environmental and governance contexts.

The innovative aspect of the methodology lies in its adaptive analytical structure. Rather than applying a fixed set of suitability criteria, the framework selects environmental indicators, spatial variables, weighting schemes, and analytical tools according to the production system under assessment. Marine low-trophic aquaculture requires the integration of hydrodynamic processes, nutrient dynamics and ecosystem interactions; organic aquaculture emphasises environmental quality, cumulative pressures and regulatory compliance; restorative aquaculture incorporates ecological status, habitat conditions and ecosystem functioning; whereas freshwater Bio-IMTA and aquaponic systems integrate land-use characteristics, water availability, renewable energy resources, logistics and proximity to markets. Species-specific ecological requirements are incorporated where appropriate through dedicated environmental datasets and modelling approaches, including Species Distribution Models.

Stakeholder participation is embedded throughout the process through participatory mapping and scenario-building workshops, integrating local knowledge, identifying spatial conflicts and supporting the co-design of sustainable aquaculture development pathways.

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Expected Outcomes and Relevance

The methodology provides a transferable framework for identifying AZA across multiple production systems while maintaining methodological consistency. By adapting spatial indicators, datasets and analytical workflows to the ecological and operational characteristics of each system, the approach supports more robust site selection and extends AZA principles beyond their traditional marine applications. The framework will be implemented and validated through multiple European case studies within DIVERSIFY4Future, covering marine, transitional and freshwater environments. The expected outcome is a decision-support methodology for public authorities, spatial planners, and the aquaculture sector to facilitate the sustainable expansion of diversified and low-impact aquaculture systems.

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MICROBIOME ATLAS OF PORTUGUESE AQUACULTURE FISH

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Although microbiome imbalance, also known as dysbiosis, is linked to several pathologies, there is limited knowledge of fish microbiomes (e.g., skin, gut, gills) compared with humans and other animals. Advanced and optimized molecular biology and bioinformatics pipelines enable microbiome characterization at taxonomic and functional levels. The study of fish microbial communities represents a non-invasive or minimal way to predict early disease symptoms and enhance fish diets. Some aquaculture practices, such as tank disinfection or antibiotic use, may not only adversely affect fish pathogens but also beneficial commensal bacteria, which could lead to a weaker fish immune response, risking species fitness.

The InitMAPAfish (Initiating the Microbiome genome Atlas of Portuguese Aquaculture FISH species) project aims to establish the first open-access Microbiome Atlas of Portuguese Aquaculture fish species, focusing on economically significant species like *Oncorhynchus mykiss*, *Dicentrarchus labrax* and *Sparus aurata*. To achieve this, optimized bioinformatic Nextflow pipelines were run (on the High Performing Computer Deucalion) using shotgun DNA and RNA sequencing data retrieved from both internal projects at CIIMAR's Aquatic Animal Health Group (A2S) and public repositories like the NCBI Short Read Archive. From here, downstream and statistical analysis (such as alpha diversity, beta diversity, and differential abundance analysis) were performed to assign taxonomic classification and functional profiles (gene families, metabolic pathways, and antibiotic resistance profile) from databases like Kraken2, HUMAnN3/ MetaPhlan, and RGI-CARD. The resulting profiles and associated metadata (including tissue type, age, and health status) will be integrated into a structured Wikibase knowledge base (MAPAfish-kb), ensuring the data is findable, accessible, interoperable, and reusable (FAIR principles) for the global research community.

The atlas enables the development of non-invasive tools for early pathogen detection, which is vital for preventing the massive economic losses caused by unpredictable disease outbreaks in industrial farming. Furthermore, InitMAPAfish provides a scientific platform to evaluate the impact of new feed formulations and functional diets on fish health, potentially reducing the industry's reliance on antibiotics. This alignment with the EU Farm to Fork strategy and UN Sustainable Development Goals underscores the project's importance in fostering resilient and environmentally friendly food production systems.

BEHAVIOURAL RESPONSES OF GILTHEAD SEABREAM (*Sparus aurata*) TO THE ADMINISTRATION OF PAINFUL STIMULI

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Pain is defined as an unpleasant sensory and emotional experience associated with, or resembling, actual or potential tissue damage. It is a complex process mediated not only by physiological mechanisms, but also by psychological and social factors, all of which can influence its intensity and quality at the sensory, cognitive, and emotional level. Although there is substantial evidence available for pain in animals, their ability to *feel* pain rather than *sense* pain still elicits some confusion, probably due to the difficulty to clearly distinguish nociception (i.e. the neural detection of noxious stimuli), from pain as a conscious, affective experience. Despite all its negative connotations, pain is a critical ability for the survival of organisms in that it drives immediate responses, attention, and adaptive learning to avoid pain and promote healing. In fish, studies directly addressing pain are limited to a few species. In the present study, we evaluated the behavioural effects of acetic acid (AA) subcutaneously injected into the lips of gilthead seabream (*Sparus aurata*), an important aquaculture species. Two experiments were carried out: In Experiment 1, fish received sham handling (needle insertion), PBS (control solution), 1, 5 or 10% AA. In Experiment 2, fish received PBS, 10% AA, or 10% AA combined with lidocaine at 1 or 2 mg mL⁻¹. In both experiments, behaviour was recorded 1 h before treatment (Time 0) and at 0.5, 1.5, 3 and 6 h post-injection (p.i.). Qualitative inspection of the lips showed that 10% AA was associated with visible swelling and mouth opening movements, which were not observed in controls. 10% AA increased freezing, with greater use of the lower zone of the tank at 0.5, 1.5 and 3 h p.i. Co-administration of lidocaine reversed these behavioural changes at both doses tested. These results are in line with the responses found in other species to painful stimuli and suggest that 1) AA elicits a typical pain-like response in gilthead seabream and 2) the physiological sensing component shares the same conserved mechanisms of reception and transmission of painful signals as other vertebrates. These results add to the mounting evidence of pain in fishes and have ethical implications on the treatment of this (and other) fish species under a welfare perspective.

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LATE-WINTER TO SPRING CHANGES IN GLYCOGEN AND PROXIMATE COMPOSITION OF FARMED EUROPEAN FLAT OYSTERS *Ostrea edulis*

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Introduction

The biochemical composition of oyster tissues is influenced by seasonal changes in environmental conditions, food availability and reproductive activity. Glycogen represents the principal carbohydrate reserve in oysters and, together with tissue hydration, protein and lipid content, provides useful information on physiological condition and potential product quality. Despite the importance of the European flat oyster *Ostrea edulis* in Mediterranean shellfish aquaculture, information on short-term temporal changes in the biochemical composition of farmed oysters from eastern Adriatic estuarine environments remains limited. This study aimed to evaluate changes in glycogen and proximate composition of farmed *O. edulis* during the transition from late winter to spring in the Krka River estuary, Croatia.

Materials and Methods

European flat oysters were sampled from a farming site in the Krka River estuary on three consecutive occasions between February and April 2021. At each sampling period, eight composite samples were prepared, each comprising pooled soft tissues from seven oysters. Moisture, crude protein, total lipid and glycogen were determined in duplicate. Differences among sampling periods were evaluated statistically using one-way ANOVA followed by post-hoc comparisons. Since variation in tissue water content may influence biochemical concentrations expressed on a wet-mass basis, glycogen, protein and lipid were additionally evaluated after normalization to dry matter.

Results

A distinct temporal change in oyster tissue composition was observed between late winter and spring. Moisture content progressively decreased and was significantly lower during the final sampling period. In contrast, glycogen concentrations increased towards spring, with the highest values recorded in April. Protein content expressed on a wet-mass basis followed a similar pattern and was significantly higher during the final sampling period, whereas total lipid content remained comparatively stable throughout the study.

Normalization to dry matter substantially reduced the magnitude of temporal differences in protein and lipid content. Glycogen maintained an increasing pattern towards April, although the difference was less pronounced than when expressed on a wet-mass basis.

Discussion

The decrease in tissue moisture accompanied by increasing glycogen and protein concentrations on a wet-mass basis indicates a transition towards a tissue composition with a greater proportion of dry matter during spring. The increase in glycogen is consistent with seasonal changes in energy reserves previously reported for *O. edulis*, in which reserve accumulation and utilization are closely related to food availability and reproductive processes. However, the attenuation of differences after dry-matter normalization demonstrates that part of the apparent biochemical increase resulted from decreasing tissue hydration.

The relatively stable lipid content suggests that lipids contributed less to the observed short-term temporal variation than glycogen. Previous studies on European flat oysters have similarly demonstrated considerable seasonal variation in biochemical composition, although the magnitude and timing of these changes differ among geographical areas and populations. Such differences may reflect local environmental conditions, food availability and reproductive phenology.

The results demonstrate that glycogen and tissue hydration are sensitive indicators of short-term changes in the biochemical condition of farmed *O. edulis*. Reporting biochemical constituents on both wet- and dry-mass bases may therefore provide a more accurate assessment of temporal changes in oyster condition and potential harvest quality

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EVALUATION OF A HYDROGENATED VEGETABLE FAT AS AN ALTERNATIVE LIPID SOURCE FOR NILE TILAPIA (*Oreochromis niloticus*)

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Introduction

Lipids are essential components of aquaculture diets, providing a concentrated source of energy and essential fatty acids. Fish oil has traditionally been an important lipid source in aquafeeds; however, its limited availability and the continued growth of aquaculture have increased interest in alternative lipid sources (Nasopoulou and Zabetakis, 2012). Vegetable oils and fats can reduce dependence on finite marine resources and provide greater flexibility in feed formulation, with numerous studies demonstrating their potential to partially or even fully replace conventional dietary oils without compromising fish performance (Alhazzaa et al. 2019; Qian et al. 2024).

Besides their nutritional value, technological properties such as oxidative stability, handling, and consistent quality are relevant for the selection of dietary fats. Refining provides standardized fat quality, while hydrogenation increases oxidative stability and results in a solid fat with favorable handling characteristics. BEWI-SPRAY (BEWITAL agri GmbH & Co. KG, Germany) is a refined and hydrogenated triglyceride in powder form developed as an alternative lipid source for animal nutrition.

The present study evaluated BEWI-SPRAY (BS) in diets for Nile tilapia (*Oreochromis niloticus*) and investigated whether 33% or 66% of a soybean and sunflower oil mixture could be replaced without negatively affecting growth performance, feed utilization, survival, or apparent nutrient digestibility.

Materials and Methods

Three isonitrogenous, isoenergetic, and isolipidic diets were formulated containing approximately 42% protein, 21 MJ/kg gross energy, and 12% lipid. A 1:1 mixture of soybean and sunflower oil served as the lipid source in the control diet. This mixture was replaced by BEWI-SPRAY at 33% (BS33%) or 66% (BS66%). Nile tilapia with an initial body weight of approximately 22.4 g were randomly distributed into 80 L tanks at 15 fish per tank. Each dietary treatment was tested in triplicate for 56 days. Fish were fed twice daily to apparent satiation.

Growth performance, feed utilization, survival, and apparent digestibility coefficients of dry matter, crude lipid, protein, organic matter, and energy were evaluated. Chromic oxide was included as an inert marker for determination of apparent nutrient digestibility (Furukawa and Tsukahara 1966; NRC, 2011). Treatment effects were analyzed using one-way ANOVA, and linear and quadratic responses to increasing BS inclusion were assessed using polynomial contrasts. Differences were considered significant at $P < 0.05$.

Results

Dietary inclusion of BEWI-SPRAY fat powder had no significant effect on growth or feed utilization parameters ($P > 0.05$). Final body weights were 90.02, 89.25, and 91.98 g/fish for the control, BS33%, and BS66% treatments, respectively. Specific growth rate ranged from 2.46 to 2.51%/day, while feed conversion ratio ranged from 0.925 to 0.974 without significant differences ($P > 0.05$). Protein efficiency ratio and condition factor were likewise unaffected by dietary treatment. No mortality occurred during the experimental period, resulting in 100% survival in all groups.

Likewise, no significant differences were observed in the apparent digestibility coefficients of dry matter, crude lipid, protein, organic matter, or energy ($P > 0.05$). Crude lipid digestibility was 92.4%, 92.9%, and 92.7% in the control, BS33%, and BS66% treatments, respectively. Protein digestibility remained above 96% in all treatments. Increasing BS inclusion resulted in neither significant linear nor quadratic responses for growth, feed utilization, or apparent nutrient digestibility.

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Discussion

The results demonstrate that BEWI-SPRAY fat powder can replace up to 66% of the soybean and sunflower oil mixture without adversely affecting growth performance, feed utilization, survival, or apparent nutrient digestibility in Nile tilapia. Particularly noteworthy is the consistently high apparent lipid digestibility, which remained above 92% across all treatments. Even at the highest inclusion level, lipid digestibility was 92.7% compared with 92.4% in the control diet. This indicates that the refined and hydrogenated vegetable fat was efficiently digested and utilized despite its different fatty acid composition and physical characteristics compared with conventional liquid vegetable oils.

These findings support BEWI-SPRAY fat powder as a suitable alternative lipid source for Nile tilapia diets. The use of refined and hydrogenated vegetable fats may provide additional flexibility in aquafeed formulation while reducing dependence on conventional lipid sources. Their standardized quality and increased oxidative stability may also offer technological advantages during feed production and storage. Furthermore, the inclusion of lauric acid provides an interesting nutritional characteristic that warrants further investigation. Overall, the present results show that BEWI-SPRAY Boost RS can partially replace conventional vegetable oils while maintaining growth, feed efficiency, and nutrient utilization in Nile tilapia.

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INTEGRATED MULTI-TROPHIC AQUACULTURE (IMTA) OF *Chondrosia reniformis* FOR MARINE COLLAGEN PRODUCTION

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Introduction

The global demand for collagen is steadily rising, with a projected compound annual growth rate (CAGR) of 6.52% through 2027. Owing to its high biocompatibility, notable bioactivity and low antigenicity, collagen is a versatile protein widely utilized across multiple industries. Its applications span pharmaceuticals and biomedical products, as well as nutritional supplements, cosmetics, and food. While collagen derived from terrestrial animals is abundant, concerns related to disease transmission have limited its suitability for human use. As a result, marine-derived collagen has emerged as a promising alternative, with marine sponges representing a particularly valuable and sustainable source. In this study *Chondrosia reniformis* is of particular interest due to the high concentration (> 30%) of type I collagen in the sponge biomass (Pozzolini et al. 2012) and the absence of any hard structures (spicules).

A primary bottleneck in utilizing sponge collagen commercially is securing a dependable supply of sponge biomass. Cultivating *C. reniformis* in mariculture shows promise for securing a steady, eco-friendly supply of sponge biomass for collagen extraction (Orel et al, 2021), but the success of sponge farming is influenced by naturally fluctuating conditions such as temperature, light, current, and turbidity. Predation, disease, and fouling can also harm this species in mariculture. In closed, recirculating aquaculture systems, optimal species-specific cultivation conditions could be established, enabling sustainable, reliable, and location-independent production of sponge biomass and, as well as the potential for a targeted maximization of 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. 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). 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.

This study focuses on the joint, almost automated cultivation of the sponge species *C. reniformis* alongside commercially relevant macroalgae and fish within the framework of an IMTA approach.

Materials and methods

***Chondrosia reniformis*:** The sponge specimen, obtained by diving from the Adriatic Sea (by the University of Ancona, UNIVPM), were transported to the recirculation aquaculture system at the Alfred Wegener Institute, Bremerhaven, Germany. After an acclimatization time of one month in artificial seawater, sponge specimen were fragmented in pieces of ca. 1x1x1 cm and were distributed as followed: a) shelf system (6x 200l tanks): four fragments were placed on the clay dish and randomly distributed on the bottom in each of the six tanks b) circular round tank (750 l): Inside the round tank, there are four custom-designed stands, each consisting of a granite stone with four horizontal struts; fragments rest on a clay bowl within a metal basket supported by these struts. Fragments were fed once daily with a self-designed powder feed (project AkPhaKol), which is dissolved before feeding. A specialized syringe pump was designed for automated feeding. Additionally, to the experiment on the growth behavior of the sponge fragments during the IMTA assembly, two other experiments on the targeted increase of the collagen content in the IMTA assembly were conducted: 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 and growth is calculated in measured volume (cm³) at the beginning and end of each experiment. The collagen content of sponge fragments was documented at the beginning and at the end of each experiment as a percentage of dry weight.

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***Ulva lactuca*:** Free-floating algae were obtained from the macroalgae project, called Novafoodies, conducting at the AWI. After a starting material of 100 g was distributed in each of the four cascaded tanks (each 0.06 m³), the algae-system was connected to the sponge cycle. The nutrient concentrations (ammonium, nitrite, nitrate, phosphate) in the sponge-algae system were regularly monitored. The growth and photosynthetic efficiency parameters of the algae are measured every four weeks. To prevent nutrient deficiency in the algal biomass, a special algae fertilizer is initially added daily.

***Dicentrarchus labrax*:** Juveniles of European sea bass were obtained from a commercial French fish farm. After an acclimation period and removal of group 0 for measurement of length, weight, and health status, the fish tank (750 l) was connected to the sponge-algae-system. The experiment started with 400 sea bass with a size of 14 g and 11 cm in length. In the ongoing study, the quantity and size of the feed must be regularly adjusted to the growth of the fish. To maintain the stocking density, fish must be removed from the tank at regular intervals; these fish are subsequently examined to assess their size (length and weight) and health status.

To ensure consistent conditions for the IMTA approach with sponge algae and fish, the following conditions were continuously evaluated: Stocking size and density of fish and algae, documentation of health status and feed availability for the sponge tank: The amount of feed available in form of feces and fish food residues is documented. Additionally, water quality (ammonia, nitrate, nitrite, and phosphate levels) is monitored.

Initial phase and experimental procedure of the polyculture system

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 supplementary and constantly available food for the sponges together with the daily feed.

Additional experiments to increase the collagen content were conducted on a laboratory scale in a further approach. Various solids and solutions were tested, which intended to increase the collagen content in sponge fragments. 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. The co-cultivation of sponges with other economically valuable species in an integrated multi-trophic aquaculture system is expected to provide species-specific water quality conditions that support high fitness and growth of sponge fragments. 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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ENHANCING AQUACULTURE COMPETITIVENESS THROUGH VERTICAL VALUE-ADDED PROCESSING; OPPORTUNITIES AND CONSIDERATIONS FOR PRODUCERS

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Expanding globalization of seafood markets continues to place increasing competitive pressure on all sectors of aquaculture production. One strategy with significant potential to improve producer profitability is the vertical integration of value-added processing. Vertical value-added approaches enable aquaculture producers to differentiate products, offer consumer-preferred “easy-to-prepare” or ready-to-eat options, and capture processing margins beyond the farm gate.

Importantly, value-added processing also presents opportunities to enhance and communicate product reliability, food safety, and consistent high quality—critical elements for building consumer trust and brand recognition. Additional benefits may include improved utilization of coproducts, contributing to greater overall system efficiency and revenue diversification.

This presentation will review examples of vertical value-added processing and discuss market-alignment considerations relevant to producers. Examples from multiple aquaculture sectors will be used to illustrate existing value-added products and integration strategies, providing practical insights for evaluating the economic viability of vertical integration opportunities.

LEVERAGING DATA TO REDUCE FISH STRESS & MORTALITY

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Sensor technology has improved, reducing costs and creating richer data streams on more parameters. This presentation will cover two case studies where clients leveraged sensor data to make dramatic improvements in farm performance.

Case Study 1: Topian addresses thermal stress

Topian Aquaculture, a farmer of sea bass and bream in the Red Sea, wanted to produce the higher-value greater amberjack (*Seriola dumerili*), but several cohorts had to be abandoned after high summer temperatures led to mass mortality. They looked to Innovasea, which helped them by deploying temperature sensors to capture their thermocline. Innovasea then deployed two submersible pens, which Topian operated below the thermocline for the warmest four months of the year, reducing the experienced temperature by ~2°C and alleviating thermal stress for the fish. Topian finished their first *Seriola* harvest in Dec 2025 with 12.7% mortality.

Case Study 2: Cooke

In 2018, Cooke bought an O₂ system to reduce the impact of hypoxia events and increase the available feeding windows. They used Innovasea's aquaMeasure sensors in each pen and observed striking differences in the oxygen levels in each pen, before supplementation. They wanted a system to control oxygen flow on a pen-by-pen basis. Innovasea created aquaControl, which enables farmers to monitor and control oxygen and airflow to each pen. Cooke was able to reduce their O₂ needs dramatically without compromising in-pen conditions.

NUCLEOTIDE-BASED FORMULATION ENRICHED WITH SEAWEED MEAL ENHANCES GILTHEAD SEA BREAM (*Sparus aurata*) HEPATOCYTE PROLIFERATION AND MODULATES LPS-INDUCED INFLAMMATORY SIGNALING *IN VITRO*

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Background and objective

Dietary nucleotides are increasingly recognized as functional feed ingredients that enhance growth, metabolic resilience, and immune function (1). Their importance in aquaculture is rising as climate change, environmental variability, and intensive farming practices represent greater physiological and immunological stress on cultured fish. Although nucleotide-based formulations enriched with seaweed meal (NUSW) are used in Pacific white shrimp (2-4), evidence in marine finfish remains limited. This study assessed the effects of NUSW on gilthead sea bream (*Sparus aurata*) hepatocyte viability and inflammatory gene responses following a lipopolysaccharide (LPS) challenge *in vitro*.

Methods

Primary hepatocytes from gilthead seabream were cultured *in vitro* and exposed to a NUSW product (Nucleoforce® Immunity, Bioiberica SAU, Spain) at concentrations of 0.042, 0.014, and 0.0046 mg/mL, corresponding to practical feed inclusion levels. After 24 h of incubation, cell viability was assessed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. For immunomodulatory assessment, hepatocytes were challenged with bacterial LPS (50 µg/µL) for 5 h, and cytokine gene expression (IL-1β, TNF-α, IL-15) was quantified by qPCR.

Results

NUSW showed no cytotoxicity at any concentration tested, maintaining hepatocyte viability above 80% after 24 h. At 0.042 mg/mL, NUSW significantly increased cell viability compared to the negative control (26.03 ± 5.27%) (Figure 1), suggesting a proliferative and/or cytoprotective effect at a nutritionally relevant feed-equivalent inclusion level (166.6 ppm in feed). Following LPS challenge, NUSW modulated inflammatory gene expression, with stimulatory effects on TNF-α, a cytokine previously associated with nucleotide-mediated immunomodulation in mammals. Although functional implications in fish require further studies, these results indicate that NUSW may influence inflammatory signaling under stress-relevant conditions in fish.

Conclusions

These *in vitro* results indicate that NUSW (Nucleoforce® Immunity) can enhance hepatocyte viability and proliferation while modulating inflammatory responses under LPS challenge, thus supporting its potential to improve fish resilience and contribute to more sustainable aquaculture practices. By strengthening physiological robustness and immune responsiveness, such functional feed ingredients align with global priorities to enhance production efficiency, mitigate stress, and reduce reliance on antimicrobial interventions in aquaculture systems.

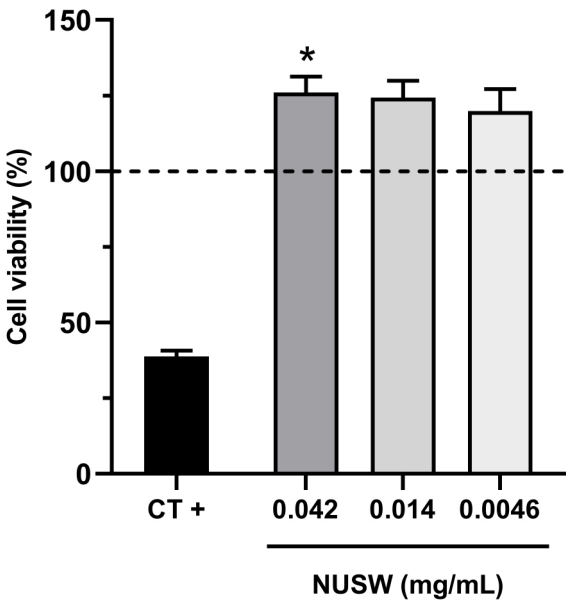


Figure 1. Percentage of cell viability of gilthead sea bream hepatocytes at day 3 after 24 h incubation with a Nucleoforce® Immunity product (NUSW) at three 3-fold serial dilutions or 10% DMSO (positive toxicity control, CT +). Data are shown as mean ± SEM and presented as fold change relative to the negative control condition (dashed line, 100%) (n = 6 independent cell cultures). Significant differences were determined using the T3 Dunnett test and are indicated by asterisks (*p* < 0.05).

Acknowledgement

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FROM FOOTPRINT TO RECOVERY: GEOCHEMICAL AND EDNA INDICATORS OF ATLANTIC BLUEFIN TUNA FARMING IMPACTS ON THE SEABED

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Introduction

Atlantic bluefin tuna farming is associated with local organic enrichment of sediments, shifts in redox conditions and degradation of benthic communities, particularly beneath and around net-pen structures. Although environmental regulations increasingly require evidence of habitat recovery after farm cessation, the trajectory and timescale of benthic recovery under different hydrodynamic and depositional settings remain poorly resolved. This study aimed to characterise the spatial footprint of tuna farming and quantify benthic recovery using a combination of basic geochemical indicators and eDNA-based biotic indices at an active farm, two abandoned tuna farm sites and a reference site in the central Adriatic Sea.

Materials and Methods

One active tuna farm, two abandoned sites at different recovery stages (10 and 15 years after cage removal) and one reference location unaffected by aquaculture were sampled. At each site, nine stations were established along a 900 m radial transect, covering a gradient from directly beneath the former or current cages to off-farm reference areas beyond detectable organic enrichment. Surface sediments were analysed for total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP) and redox potential (Eh) as key indicators of organic loading and reduced conditions. In parallel, eDNA metabarcoding was applied to sediment samples to generate MOTU matrices, from which taxonomic richness and gAMBI-like biotic signals, a genetic derivation of the classical AMBI index, were derived. Spatial patterns in geochemical variables and eDNA-based indices were evaluated in relation to distance from cages and site type.

Results and Discussion

At the active farm, sediments beneath cages showed elevated TOC, TN and TP, strongly reduced Eh and benthic communities dominated by opportunistic polychaete taxa, including *Capitella*, *Prionospio*, *Neanthes* and *Mediomastus*, consistent with heavily impacted softbottom habitats. High gAMBI values confirmed severe ecological disturbance and demonstrated that the eDNA-based index effectively captured the footprint of intense organic enrichment. The 10-year fallowing site showed partial recovery, with reduced TP and improved redox conditions but still elevated organic matter relative to the reference site. At the 15-year site, TOC, TN, TP and Eh largely converged towards reference levels, and eDNA-based indices indicated advanced biological recovery approaching the undisturbed status of the control location. Across all sites, gAMBI derived from eDNA data closely tracked the gradient of organic enrichment and clearly separated active, recovering and reference conditions. The combined use of TOC, TN, TP, Eh and eDNA metabarcoding thus provides a practical, sensitive framework to map tuna farm impacts and quantify benthic recovery after production stops. The progression from strongly impacted sediments at the active farm to near-reference conditions at the long-fallowed site highlights both the recovery potential of benthic habitats and the importance of site-specific conditions and farm history in determining recovery timescales.

Acknowledgment

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PROTECTIVE EFFICACY OF A LIPOSOME-BASED ORAL VACCINE AGAINST *Streptococcus iniae* IN KOREAN ROCKFISH (*Sebastes schlegelii*)

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Introduction

Korean rockfish (*Sebastes schlegelii*) is a widely cultured marine fish species in Northeast Asia and is the second most produced marine aquaculture species in Korea, making it economically important. Injectable vaccines are commonly used; however, their application in aquaculture cage systems is limited due to handling constraints. Therefore, the development of oral vaccines, which enable easy and convenient administration without handling stress, is essential for efficient mass immunization. This study evaluated the immunogenicity, protective efficacy, and safety of a liposome-based oral vaccine against *Streptococcus iniae* infection.

Materials and Methods

Formalin-killed *S. iniae* was prepared by culturing bacteria in brain heart infusion broth supplemented with 1.5% sodium chloride and inactivated with 0.1% formalin. The antigen was encapsulated in lecithin-based liposomes using a microfluidizer and coated with chitosan. The vaccine was incorporated into feed at 10, 20, and 40 mg/fish. Korean rockfish were orally immunized for 6 days and reared at 21°C for 14 weeks. Antibody titers and biochemical parameters were analyzed. At 4 weeks post-vaccination, fish were challenged with *S. iniae* and mortality was monitored for 24 days at 25°C. Histopathology was conducted to evaluate vaccine safety.

Results

Antibody titers increased progressively until 10 weeks post-vaccination and declined thereafter. At week 10, titers were up to 4.75-fold higher than those of the control group, showing a clear dose-dependent response. The proportion of antibody-positive fish exceeded 60% from weeks 2 to 12 and peaked at 86.7% at week 4, indicating effective induction of population-level immunity. No significant differences in blood biochemical parameters and histopathological findings were observed compared with the control group, indicating no adverse effects of the vaccine.

Conclusion

The liposome-based oral vaccine showed effective protection against *S. iniae* infection without adverse effects. These results suggest that this vaccine is a promising strategy for disease control in aquaculture.

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STANDARDIZING IMMUNOLOGICAL TOOLS FOR *Penaeus vannamei* HEALTH ASSESSMENT

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Introduction

Shrimp farming plays a vital role in global aquaculture, with the whiteleg shrimp (*Penaeus vannamei*) being the most widely farmed species. However, disease outbreaks continue to cause significant economic losses, highlighting the need for reliable indicators to assess shrimp health and immune status (Flegel et al., 2019). Immune parameters in *P. vannamei*, including hemocyte counts, phenoloxidase (PO) activity, and antimicrobial peptide (AMP) gene expression, are commonly used to evaluate immune function (Tassanakajon et al., 2013; Xu et al., 2014). However, reported values vary widely across studies due to differences in sampling, handling, and shrimp physiological conditions. This variability limits the establishment of reliable baseline values and complicates comparisons between laboratories and production systems. Therefore, this study aims to standardize methods and establish reference values for key immune parameters in healthy *P. vannamei*, including total (THC) and differential hemocyte counts (DHC), PO activity, and AMP gene expression. Establishing consistent reference ranges for these parameters will support reliable health assessment and improve disease monitoring in aquaculture systems.

Materials and Methods

Shrimp (average body weight, 10 g) at the intermoult stage were selected for sampling. Hemolymph samples were collected using a well established in-house procedure under sterile conditions to minimize hemocyte activation and subsequent hemolymph coagulation. THC was determined through manual counting using a hemocytometer, while DHC was performed using optimized staining methods to differentiate hemocyte subpopulations. In parallel, high-throughput profiling was conducted using flow cytometry to characterize hemocyte subpopulations and validate manual counting results. Functional immune parameters were assessed through optimization of PO activity assays. Molecular immune responses were evaluated through RT-qPCR, targeting AMP genes.

Results and Discussion

Preliminary results showed that THC averaged approximately 10^7 hemocytes mL^{-1} hemolymph ($n > 100$ shrimp from different batches), which is at the upper range of values commonly reported in the literature (10^5 – 10^6 hemocytes mL^{-1} hemolymph) (Yildirim-Aksoy et al., 2022; Huang et al., 2013). Reported hemocyte counts vary widely, possibly due to differences in shrimp handling, hemolymph collection, and hemocyte sampling procedures. Lower counts observed in other studies may be mainly attributed to suboptimal hemolymph collection techniques and subsequent clotting. To reduce these sources of variation, standardized hemolymph collection and handling protocols were applied in this study. DHC showed improved morphological differentiation using optimized staining, addressing common inconsistencies in hemocyte classification. Flow cytometry results were comparable to manual counting, supporting its use as a reliable and high-throughput method for standardized immune monitoring and reducing repetitive manual labor.

PO activity measurements are frequently influenced by differences in assay parameters, such as substrate and incubation time. In this study, PO assays are being optimized under standardized conditions to improve consistency. Similarly, AMP expression can vary due to PCR efficiency and normalization methods. The verified amplification efficiency ($E = 88$ – 92%) indicates reliable assay performance, although further validation is required to establish consistent reference ranges for these functional immune parameters.

Overall, inconsistencies in immune parameter measurements highlight the need for standardized analytical procedures to ensure comparable and reproducible results. Once validated, these standardized procedures and derived immune parameter reference values will serve as practical diagnostic indicators for shrimp health monitoring. Similar to blood tests in human and veterinary medicine, routine immune assessment may enable early detection of physiological stress or immune suppression and support proactive health management in shrimp aquaculture systems, while reliable reference values for healthy shrimp would strengthen research on diseases and treatment strategies.

(Continued on next page)

Acknowledgement

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REDUCED WATER EXCHANGE DOES NOT COMPROMISE GROWTH AND WELFARE OF ATLANTIC COD JUVENILES IN RECIRCULATING AQUACULTURE SYSTEM

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Introduction

Atlantic cod (*Gadus morhua*) is a commercially important species in Norway, but its production has traditionally relied on flow-through and sea-based systems (Puvanendran et al., 2022; Nardi et al., 2021). Recirculating aquaculture systems (RAS) offer improved control of environmental conditions, biosecurity and water quality, providing opportunities for more predictable land-based cod production (Wold et al., 2014; Badiola et al., 2017). However, water exchange is an important operational parameter because increasing exchange rates can improve the dilution of metabolites and dissolved compounds while simultaneously increasing water demand, pumping and treatment requirements. Identifying the lowest effective water exchange rate is therefore important for developing water- and energy-efficient RAS production protocols for Atlantic cod juveniles. This study evaluated the effects of reduced water exchange on growth performance, water quality and welfare of Atlantic cod juveniles reared in RAS.

Materials and Methods

Atlantic cod juveniles with an initial body weight of 137–138 g were stocked at 75 fish per 500 L SingleRAS tank in triplicate. Two water exchange regimes were tested: low water exchange (LWE; 500 L kg⁻¹ feed day⁻¹) and high water exchange (HWE; 1000 L kg⁻¹ feed day⁻¹). Fish were reared for seven weeks at 12 °C, salinity 34‰, pH 7.9, dissolved oxygen 8.6 mg L⁻¹ (>95% saturation), total gas pressure (TGP) 99.5% and, 24-hour photoperiod. Fish were fed a commercial diet (Skretting Amber Fortuna) using automatic belt feeders with hourly feeding. Growth performance, feed conversion ratio, survival, water quality and external welfare indicators were monitored throughout the experiment.

Results

Reducing water exchange from 1000 to 500 L kg⁻¹ feed day⁻¹ did not significantly affect growth performance, feed conversion or survival. Final body weight was 187.7 ± 6.4 g in LWE and 194 ± 2.3 g in HWE, while specific growth rate was 0.61 ± 0.07 and 0.68 ± 0.02% day⁻¹, respectively. FCR was 1.54 ± 0.12 in LWE and 1.37 ± 0.09 in HWE, and survival remained high at 96.4 ± 0.8% and 97.3 ± 1.3%, respectively.

Core water quality parameters remained stable in both treatments. TAN, nitrite and CO₂ did not differ significantly between treatments. However, nitrate increased significantly under LWE, reaching 76.1 ± 1.9 mg L⁻¹ compared with 40.8 ± 6.4 mg L⁻¹ in HWE. Total organic carbon and dissolved organic carbon were also significantly higher under LWE, at 21.07 ± 0.29 and 18.63 ± 1.34 mg L⁻¹, respectively, compared with 15.5 ± 0.53 and 12.3 ± 0.17 mg L⁻¹ in HWE. Despite the increased dissolved organic load, suspended solids and turbidity did not differ significantly between treatments. UV transmittance decreased to 53.2% under LWE compared with 66.1% under HWE, indicating a potential reduction in UV disinfection efficiency at lower water exchange.

External welfare scoring indicated generally low levels of welfare-related abnormalities in both treatments, with few eye, opercular, jaw, and skeletal deformities. Mandibular deformities were observed more frequently than maxillary deformities. Lower jawbone protrusion and caudal haemorrhaging were observed in some fish, while nephrocalcinosis was also observed in some individuals, although its prevalence was significantly lower.

Conclusion

This study demonstrates that Atlantic cod juveniles can be successfully reared at 500 L kg⁻¹ feed day⁻¹ without compromising growth, survival or welfare. This supports the potential of RAS for efficient land-based production of Atlantic cod (Badiola et al., 2017; Puvanendran et al., 2022). However, reduced water exchange increased nitrate and dissolved organic matter accumulation and reduced UV transmittance, highlighting the importance of effective water treatment and organic matter management in low-exchange RAS (Wold et al., 2014).

(Continued on next page)

Acknowledgment

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DIETARY POLYPHENOLIC EXTRACTS HAVE ALTERNATIVE PATHS TO MODULATE REDOX AND METABOLIC RESPONSES IN TURBOT POSTLARVAE

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Introduction

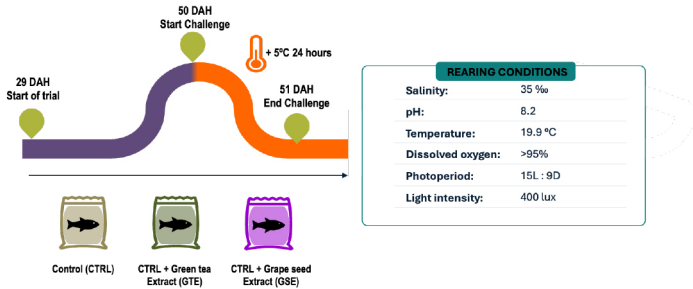
Nutritional modulation occurring during critical windows of development, may have long-term consequences on somatic structures, physiological functions or metabolic status of the organism (Gatlin, 2003). To identify optimum dietary formulations, applying dietary modulation concepts to fish nutrition offers opportunities to tailor specific metabolic pathways and physiological functions in farmed fish species (Canada et al., 2019). There has been a growing interest in dietary inclusion of plant-derived extracts containing a wide spectrum of bioactive molecules that act as appetite enhancers, growth promoters and immunostimulants in fish (Xavier et al., 2022). Green tea (*Camellia sinensis* L.) and grape seed (*Vitis vinifera* L. ssp. sativa) are two plants known for their abundant content and diversity of polyphenols. Several biotic (age, species) and abiotic factors (temperature, diet) can modulate the antioxidant defences and as a consequence, the oxidative status of the animals. Therefore, the present study was aimed to evaluate if dietary polyphenolic extracts (green tea and grape seed) supplementation modulates growth performance, antioxidant status and metabolic responses of *Scophthalmus maximus* postlarvae and help fish to cope with a stressful event.

Materials & Methods

Turbot (*Scophthalmus maximus*) postlarvae (initial dry weight: 22.6 ± 8.0 mg) were reared in a recirculating aquaculture system (RAS) in triplicate. From 29 to 51 days after hatching (DAH), fish were fed one of three diets: a control (CTRL), and the CTRL supplemented with either green tea extract (GTE) or grape seed extract (GSE). At 50 DAH, post-larvae were subjected to a 24-hour acute thermal challenge ($+5^{\circ}\text{C}$) to assess resilience. Growth performance was recorded at 50 DAH. Biochemical markers, including superoxide dismutase (SOD) and catalase (CAT) activities, as well as lipid peroxidation (LPO), were analysed alongside the expression of genes involved in antioxidant defence (*sod2*, *cat*, *gpx4*, *nrf2*), cellular stress (*hsp60*, *hsp70*, *sirt3*), digestive capacity (*tryp*, *pept1*, *lat1*), lipid metabolism (*aco*, *fasn*, *fabp2*, *cpt1*), gut integrity (*cldn15*, *tjp1*, *muc2*), mitochondrial regulation (*atp5f1a*, *ppargc1a*, *opa1*, *ucp2*), and nitrogen metabolism (*gdh*, *glul*, *got2*) under both standard and challenged conditions.

Results & Discussion

Dietary treatments did not affect growth (352.7 ± 11.3 mg; 46.8 ± 0.18 mm). However, survival was lower in GTE and GSE groups (69.0%) compared to CTRL (77.7%), suggesting that although bioactive, inclusion levels may require refinement for post-larvae.



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Distinct molecular strategies emerged between extracts. Under standard conditions, GTE-fed larvae showed a “sparing effect” on endogenous systems, with significantly lower expression of *cat*, *nrf2*, and *sirt3*. Conversely, GSE-fed larvae exhibited “molecular readiness”, significantly upregulating genes related to gut integrity (*tjp1*), mitochondrial dynamics (*opa1*), and amino acid transport (*lat1*).

Following the thermal challenge, GTE larvae exhibit lower LPO levels than CTRL, likely through direct radical-scavenging rather than enzymatic activation. In contrast, GSE-fed larvae displayed a robust, proactive response to stress, with significant upregulation of antioxidant (*cat*, *gpx4*), chaperoning (*hsp70*), digestive (*tryp*), and metabolic (*atp5f1a*, *got2*, *gdh*) pathways compared to CTRL. These results indicate that while GTE acts as a passive protector, GSE functions as a potent priming agent, enhancing the metabolic and redox machinery required to meet increased physiological demands during environmental stress.

Conclusion

Dietary polyphenolic extracts induced distinct physiological strategies in turbot post-larvae without affecting growth. Under thermal stress, GTE-fed larvae relied primarily on direct radical scavenging, effectively mitigating oxidative damage while sparing endogenous defences. Conversely, GSE promoted a broad molecular priming effect, activating a robust metabolic and antioxidant response to meet increased physiological demands. The lower survival rates indicate that while these extracts offer significant redox advantages, inclusion levels must be carefully balanced to avoid metabolic trade-offs or potential toxicity during early ontogeny.

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DIETARY SUPPLEMENTATION OF *Azolla pinnata* AND *Bacillus subtilis* IMPROVES GROWTH PERFORMANCE AND HEALTH INDICES OF *Cyprinus carpio* (LINNEAUS, 1758)

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The demand for sustainable aquaculture has led to the exploration of alternative feed ingredients and functional additives that can enhance fish growth, improve health while reducing reliance on fish meal. *Azolla pinnata*, a fast-growing free-floating aquatic fern commonly found in the Indian subcontinent, has gained attention as a potential feed ingredient due to its high protein content, balanced amino acid composition, and rapid biomass accumulation. In addition, probiotics such as *Bacillus subtilis* are widely recognized for their ability to improve gut microbial balance, enhance nutrient digestion, and promote better growth performance in cultured fish. The study evaluated the combined effects of *A. pinnata* and *B. subtilis* supplementation on the growth performance, digestive physiology and serum biochemistry of *C. carpio* fingerlings under controlled laboratory conditions for 90 days. Five experimental diets were formulated- control (T_0); *A. pinnata* 1.5g/Kg alone (T_1); *A. pinnata* (1.5g/Kg) with *B. subtilis* at 1×10^5 (T_2); 1×10^6 (T_3); and 1×10^7 CFU/mL (T_4).

Fish fed on diets supplemented with *A. pinnata* and *B. subtilis* showed improved responses compared to the control, with the T_3 diet (*A. pinnata* 1.5 g kg⁻¹ + *B. subtilis* 1×10^6 CFU mL⁻¹) showing significantly better growth in terms of higher % weight gain (87.20%), improved FCR (2.12), and survival rate ($p < 0.05$). Additionally, digestive enzyme such as protease (82.35 ± 1.95 U/mg), lipase (3.79 ± 0.06 U/mg), amylase (0.313 ± 0.007 U/mg), cellulase (0.131 ± 0.02 U/mg) activities were significantly higher than the control, indicating enhanced digestion of proteins, lipids, carbohydrates, and fibre. Serum biochemical indices significantly improved across all supplemented treatments, with T_3 exhibiting the most pronounced response-reduced liver enzyme (SGOT, SGPT, ALP), glucose, urea, creatinine levels, and increased total protein indicating better metabolic health of *C. carpio*. The combined dietary supplementation of *A. pinnata* and *B. subtilis* significantly improved growth and health in *C. carpio* with T_3 diet being most effective; this combination offers sustainable approach to enhance productivity and fish health in carp aquaculture.

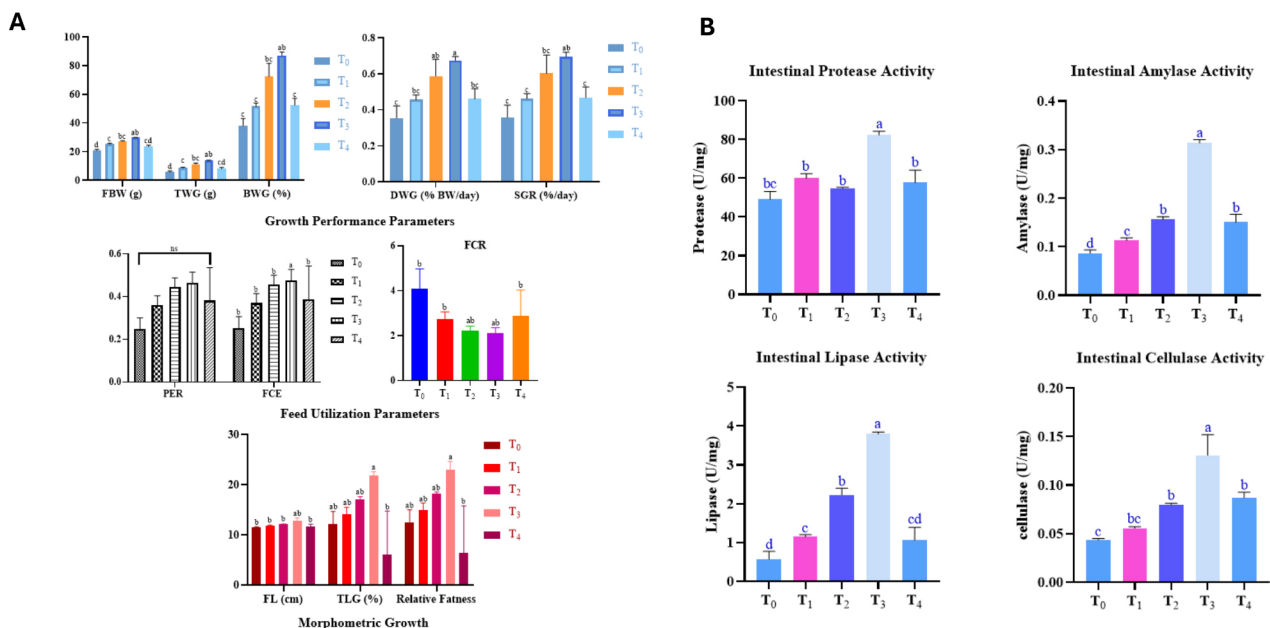


Figure 1: Treatment-dependent alterations in (A) growth performance and (B) intestinal digestive enzyme activities of *C. carpio* fingerlings fed diets supplemented with *A. pinnata* and varying concentrations of *B. subtilis*.

FISH OIL FINISHING IN AFRICAN CATFISH (*Clarias gariepinus*) – EFFECTS ON GROWTH, FILET FATTY ACIDS, GENE EXPRESSION, AND NITROGEN RELEASE

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Introduction

The functionally essential and human health promoting omega-3 fatty acids EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) are still primarily provided through finite marine raw materials (fish meal/oil) and supply is increasingly strained by a growing aquaculture industry. In the face of this EPA/DHA scarcity (Glencross et al., 2025; Tocher et al., 2019), the challenge is how to allocate the limited supply of these valuable fatty acids most effectively in order to a) minimize their use wherever possible while b) simultaneously ensuring nutraceutical EPA/DHA levels in the final product. In contrast to their marine counterparts, many freshwater fish possess the entire pathway for EPA/DHA biosynthesis from the C₁₈ precursor ALA (α -linolenic acid), suggesting the potential for entirely marine-ingredient-free and low EPA/DHA grow-out diets combined with EPA/DHA-containing finishing diets to achieve economically and nutritionally optimal EPA/DHA resource allocation. In this sense, a 13-week finishing diet trial was performed with African catfish (*Clarias gariepinus*), a freshwater species capable of EPA/DHA biosynthesis (Obloh et al., 2016).

Materials & Methods

Two experimental diets were formulated in an isonitrogenous and isolipidic manner (43% crude protein, 13% crude fat) on the basis of non-marine animal protein sources. Diets only differed with regard to the added oil source with the high EPA/DHA diet (HI) being based on salmon oil (8% inclusion, EPA+DHA: 4.5 g kg⁻¹ feed) and the low EPA/DHA diet (LO) based on rapeseed oil (8% inclusion, EPA+DHA: 0.5 g kg⁻¹ feed). Fish (start weight: 153 g) were reared in 12 independent recirculating aquaculture systems (1120 L system volume) for 13 weeks (12 weeks feeding + 1 week simulated purging without feeding) at 27 °C in three experimental groups ($n = 4$): HI diet for 12 weeks (HI), LO diet for 12 weeks (LO) and switch from the LO to the HI diet after the first 6 weeks of feeding (LO-HI). Apart from tracking growth performance, fish were continuously sampled (week 0, 2, 4, 6, 7, 8, 10, 12, and 13) to investigate the development of filet fatty acid profiles, hepatic expression of genes relevant in the biosynthetic pathway from ALA to EPA/DHA (*elovl2*, *elovl5*, *fads2*) and in amino acid metabolism (*glud1a* and *gpt2*), filet proximate composition and lipid classes, as well as dissolved total inorganic nitrogen excretion.

Results

Growth and feed conversion performance did not differ significantly between the HI and the LO-HI treatment (body weight gain - BWG: 645%/641%, FCR: 0.83/0.82), whereas the fish in the LO treatment showed significantly reduced performance (BWG: 555%, FCR: 0.94) as well as a significantly higher body weight loss after the purging period (4.7% vs. HI – 4.0% and LO-HI - 3.9%). The HI-fed fish recorded the consistently highest filet EPA and DHA content, reaching 3.3 mg EPA g⁻¹ dry matter (DM) and 11 mg DHA g⁻¹ DM at the end of the trial, whereas the filet EPA and DHA content of the LO-fed fish declined until week 8 before stabilizing to finally achieve 39% (1.3 mg EPA g⁻¹ DM) and 48% (5.3 mg DHA g⁻¹ DM) of the filet EPA and DHA content of the HI-fed fish. Filet EPA and DHA content in the fish from the finishing diet group (LO-HI) increased steadily from week 8 onward to achieve 75% (2.5 mg EPA g⁻¹ DM) and 77% (8.4 mg DHA g⁻¹ DM) of the respective levels found in the HI-fed fish, which were nevertheless still significantly higher. Expression of hepatic *elovl2* and *elovl5* was significantly elevated in the LO-fed fish compared to the HI-fed fish at the end of the feeding period, with *fads2* also showing a trend towards higher expression. Dissolved total inorganic nitrogen release was significantly higher in the LO group (40.4 g kg⁻¹ feed) than in both of the other groups (35.9 g kg⁻¹ feed), coinciding with significantly higher hepatic *glud1a* expression.

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Discussion

Despite the capacity of African catfish to biosynthesize EPA and DHA from ALA as previously suggested (Obboh et al., 2016) and again implied by the gene expression and fatty acid results of the present study, fish in this trial showed significantly reduced growth and feed conversion performance when fed exclusively with the low EPA/DHA diet (LO). Combining this insight with the significantly higher dissolved nitrogen release of these fish points towards the hypothesis that exceedingly low dietary EPA/DHA concentrations stimulate active EPA/DHA biosynthesis to cover physiological requirements which in turn costs additional energy, potentially supplied at least partly through amino acid catabolism, causing a reduction in overall growth performance. However, fish in the finishing diet treatment (LO-HI) performed equally well to the fish exclusively fed the high EPA/DHA diet. While not quite achieving the filet EPA/DHA levels of the latter, filet EPA/DHA levels in the finishing diet treatment still recovered significantly compared to the LO treatment. Considering the lower costs of rapeseed oil compared to salmon oil or other fish oils, this result suggests that a finishing diet strategy for African catfish could be beneficial from a feed cost perspective while at the same time enabling high product quality regarding filet EPA/DHA content.

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WATERBORNE TRYPTOPHAN REDUCES TRANSPORT STRESS IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

The transport of live fish is a critical welfare bottleneck in aquaculture, exposing fish to multiple interacting stressors, including handling, high stocking densities, feed deprivation, and deteriorating water quality (e.g., oxygen depletion and accumulation of CO₂ and ammonia). These factors can disrupt physiological homeostasis, impair immune function, and increase mortality (Portz et al., 2006), leading to welfare and economic concerns. While the stress-reducing effects of dietary tryptophan in fish are well documented (Höglund et al., 2005; Le Floc'h & Seve, 2007; Basic et al., 2013; Sahu et al., 2020; Bampidis et al., 2024), its application as a water-soluble additive during live transport has not yet been investigated. A recent study in hybrid groupers indicates that exogenous tryptophan can modulate antioxidant status, inflammatory markers, and stress hormones under simulated transport conditions (Cao et al., 2024). However, these findings cannot be transferred directly to European freshwater species, such as rainbow trout. Furthermore, there is a need to investigate effects under real transport conditions, elucidate dose–response relationships, and consider regulatory safety aspects. Tryptophan, an essential amino acid and serotonin precursor, plays a central role in modulating the stress axis (Höglund et al., 2005; Le Floc'h & Seve, 2007; Bampidis et al., 2024; Cao et al., 2024). However, dietary approaches are limited during transport, as fish are typically fasted and tryptophan is rapidly metabolized, resulting in low bioavailability under acute stress. To address this limitation, this study investigates the direct application of tryptophan in transport water to enable gill uptake and continuous exposure. This approach offers a non-invasive, residue-free strategy to improve fish welfare and a potential alternative to conventional anesthetics.

Materials and Methods

The efficacy of a water-soluble tryptophan formulation was evaluated in rainbow trout (*Oncorhynchus mykiss*) using a two-phase experimental design.

In Phase I, transport simulation experiments were conducted under controlled laboratory conditions (up to 24 h, 30 L tanks, continuous oxygenation). Fish (500–700 g) were stocked at transport-relevant biomass densities. Treatments included an untreated control and multiple tryptophan concentrations (30, 50, 70 mg L⁻¹), each with three replicates. Based on initial results, additional trials at higher tryptophan concentrations (100, 200, and 300 mg L⁻¹) were conducted to better characterize dose–response relationships and identify effective concentration ranges.

In Phase II, the optimal concentration was validated at a commercial trout farm (Zordel, Germany), using transport tanks and systems representative of industry practice (up to 8 h transport duration and continuous oxygenation).

Across all experiments, physiological stress indicators (cortisol, glucose, lactate, hematocrit), antioxidant responses (superoxide dismutase), behavioral parameters, and water quality variables were assessed.

Results

Preliminary results indicate that waterborne tryptophan can reduce physiological stress responses, with trends toward lower lactate levels in treated groups. However, at lower concentrations (30–70 mg L⁻¹), the effects were moderate and not statistically significant, likely due to relatively low overall stress levels and biomass-dependent differences in exposure. High-dose experiments (100–300 mg L⁻¹) established a clear dose–response relationship and determined effective concentrations for industry use.

These findings highlight a key limitation of both low-dose water application and dietary supplementation: insufficient effective exposure under realistic transport conditions. This supports the hypothesis that feed-based strategies are not optimal during transport due to fasting and rapid tryptophan turnover, whereas waterborne application ensures immediate and continuous availability. Importantly, no negative effects on fish behavior or water quality were observed across treatments, confirming the safety and practical applicability of the approach.

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Discussion

This study provides the first evidence that acute exposure to waterborne tryptophan can mitigate transport-induced stress in fish, highlighting its role as a regulator of stress response under fish transport conditions. By enabling immediate and continuous uptake, this approach addresses key limitations of conventional strategies, particularly the reduced effectiveness of feed-based additives during fasting periods prior to transport. The results demonstrate the potential of waterborne tryptophan as an innovative, welfare-oriented tool to improve fish resilience during transport, with no observed negative effects on behavior or water quality. In this context, it represents a promising alternative or complement to conventional anesthetics, which may show inconsistent efficacy and are often subject to regulatory constraints.

Beyond its immediate application, the approach offers a pathway to sustainable, residue-free stress mitigation strategies. In particular, the use of natural tryptophan sources, such as macroalgae (e.g., *Ulva* spp.), may further support the development of environmentally friendly solutions for aquaculture, aligning improved animal welfare with industry sustainability goals.

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WATER SOURCE MATTERS: ASSESSING RAINWATER USE IN AQUAPONIC PRODUCTION SYSTEMS

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Introduction

Aquaponic systems significantly reduce water consumption compared to conventional agriculture due to water recirculation, making them suitable for water-scarce contexts (Barbosa et al., 2015; Love et al., 2015; Maucieri et al., 2018). They also support sustainable urban food production through low land use and nutrient recycling (Benke & Tomkins, 2017; Specht et al., 2014; Geissdoerfer et al., 2017; Klinger & Naylor, 2012). However, these systems still require external water inputs, making water source a critical factor (Tyson et al., 2011). In urban contexts, decentralized solutions such as rainwater harvesting can enhance resilience and reduce reliance on centralized supply systems (Campisano et al., 2017; Fletcher et al., 2015; Pahl-Wostl, 2015). Integrating rainwater harvesting into aquaponics raises challenges related to water chemistry and system stability (Goddek et al., 2019; Tyson et al., 2011). Despite this, existing research has mainly focused on internal system processes, overlooking alternative water sources (Goddek et al., 2019; Maucieri et al., 2018). This study addresses this gap by comparing an aquaponic system supplied with treated rainwater to one using tap water, evaluating biological and environmental performance. The aim is to assess the feasibility of rainwater use and contribute to more sustainable and resilient urban agriculture models.

Materials and Methods

The experimental activity was conducted at the Urban Farming Lab (University of Salento, Lecce, Italy), using two identical recirculating aquaponic systems (RAS) (5000 L each), one supplied with treated rainwater and the other with municipal tap water. Both systems consisted of fully coupled loops including fish tanks, sedimentation units, biofilters and deep-water culture (DWC) plant beds, ensuring continuous recirculation (≈ 10 turnovers per day). Rainwater was collected, filtered with preformed biopolymer hydrogels, obtained from a chitosan/alginate matrix used in clariflocculation processes and mineralized prior to use. Two hundred and forty Nile tilapia (*Oreochromis niloticus*) were divided and reared for four months in two 1600 L tanks (120 fish in control tank with tap water, $W_i = 79.08 \pm 28.64$ g, $L_i = 16.26 \pm 2.06$ cm, and 120 fish in treatment tank with treated rainwater, $W_i = 74.59 \pm 28.52$ g, $L_i = 15.98 \pm 1.99$ cm) under controlled conditions (24 ± 0.8 °C; pH 6-7; DO ≈ 6.5 mg L⁻¹), fed with a commercial diet (Veronesi C.F.W. with 35% crude protein) via automated feeders. Lettuce (*Lactuca sativa* var. capitata) was cultivated for four weeks in DWC systems (25 plants m²) under LED lighting (16,000 lux, 12 h photoperiod). Water quality parameters (D.O., pH, nitrogen compounds, ORP, turbidity, TDS) were continuously monitored using automatic sensor-based systems, while fish growth (biometrics and somatic indices) and plant performance (morphological traits, biomass and SPAD index) were periodically assessed.

Results

The comparative analysis showed no significant differences between the rainwater and tap water systems in terms of both fish and plant performance. Fish exhibited similar final weight ($W_f = 198.42 \pm 66.99$ g for control vs 188.30 ± 64.72 g for rainwater), final length ($L_f = 21.37 \pm 2.51$ cm for control vs 20.98 ± 2.58 cm for rainwater), growth rates (specific growth rate $SGR \approx 0.68\%$ day⁻¹ for both conditions), feed conversion ratios ($FCR = 1.65$ control vs 1.69 rainwater), and condition factors ($K = 1.96 \pm 0.18$ for control vs 1.96 ± 0.18 for rainwater), with no statistically significant differences in somatic indices or survival. Likewise, four weeks lettuce production showed comparable vertical and radial growths (Figure 1A and 1B), leaf number (Figure 1C), and overall yield, with statistical tests confirming the absence of significant differences between treatments. Minor variations were observed in plant morphology, with rainwater slightly enhancing stem diameter and tap water promoting leaf number and height. Early differences in chlorophyll content (SPAD) converged over time (Figure 1D). Sensory analysis with 15 volunteer participants also revealed no detectable differences in lettuce quality (Figure 2). Overall, the results demonstrate that treated rainwater can effectively replace conventional water sources in aquaponic systems without compromising productivity, stability, or product quality.

Acknowledgment

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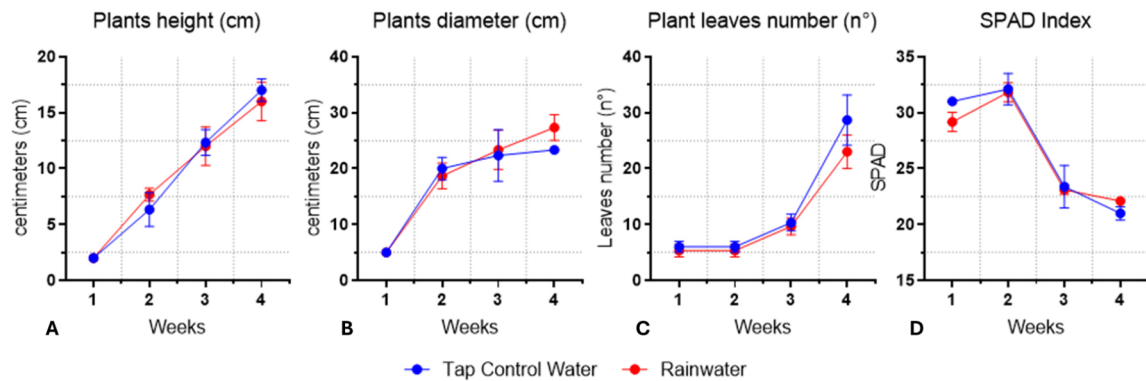


Figure 1 - graphs for A) vertical growth, B) radial growth, C) number of leaves, D) SPAD Index

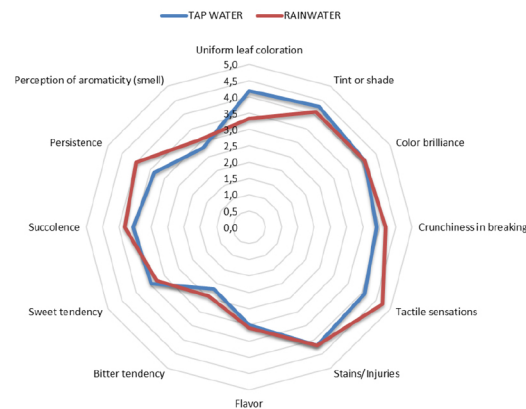


Figure 2 - Spider plot of the results obtained after the sensory analysis of aquaponic salad grown with tap water (blue) and aquaponic salad grown with rainwater.

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RECLAIMED WASTEWATER AQUAPONICS IN EUROPE: FIRST EVIDENCE OF INTEGRATED FISH PRODUCTION FROM THE “AWARE” PRODUCTION SYSTEM

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Introduction

Reclaimed water aquaculture for food production has been explored in water-scarce regions outside Europe as a strategy to enhance water efficiency and food security, although the literature remains limited and partly outdated (Kumar and Sierp, 2003; Edwards, 2000; Blumenthal et al., 2000). In Europe, wastewater reuse has traditionally focused on agricultural and environmental applications, supported by recent regulatory developments such as EU Regulation 2020/741 and circular economy strategies (European Commission, 2023; Ramm and Smol, 2024). However, integration of reclaimed water into aquaculture remains virtually absent. Historically, aquaculture operating with depurated wastewater was tested in Europe since the late 19th century, particularly in Germany, but never achieved large-scale adoption due to high operational costs, seasonal constraints, and low public acceptance.

This study presents the first fish production cycle of the first recirculating aquaponic system in Europe using reclaimed municipal wastewater. The system integrates advanced quaternary wastewater treatment with aquaponic production, demonstrating technical feasibility and addressing key barriers related to water quality, regulation, and system integration. The results contribute to the development of circular water-food systems in Europe.

Materials and Methods

The experimental activity was conducted at the “AWARE” production system (Castellana Grotte, Bari, Italy), a recirculating aquaponic system (RAS) fully supplied with treated municipal wastewater. The system (Green in Blue, Barcelona, Spain) operates as a closed-loop unit integrating fish and plant production, consisting of two fish tanks (2.7 m³ each; 5.4 m³ total) and three deep water culture (DWC) hydroponic units, each with a cultivation area of 7.5 m² (22.5 m² total). The overall system water volume is 12 m³. Water circulation is driven by a digitally controlled pump (Peraqua E.Pro) that draws water from the sump tank and passes it through a biofiltration unit, where suspended solids are retained and nitrogen is biologically converted from ammonia to nitrite and subsequently to nitrate.

A total of 150 Nile tilapia (*Oreochromis niloticus*) were reared over a nine-month production cycle in one of the two fish tanks using treated wastewater. Initial mean fish weight and length were 106.6 ± 25.5 g and 16.4 ± 1.4 cm, respectively. Fish were maintained under controlled conditions (pH 6-7; dissolved oxygen ≈ 6.5 mg L⁻¹) and fed a commercial floating diet (Dibaq Tilapia Feed, 2 mm, 35% crude protein) using automated feeding systems adjusted monthly.

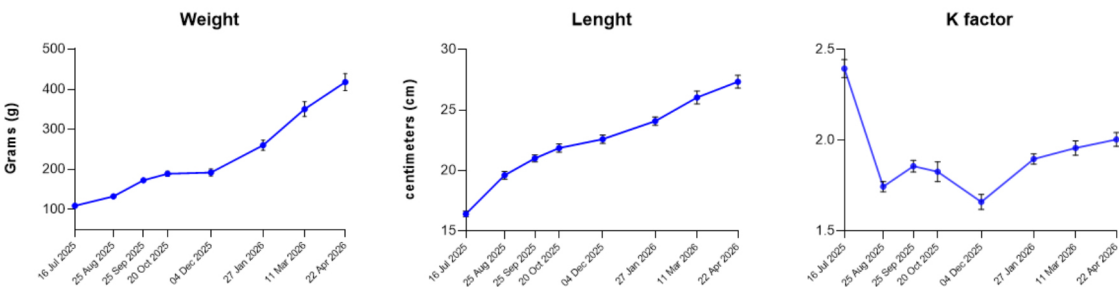


Figure 1 - Growth parameters in tilapia reared in the new AWARE aquaponic system.

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Simultaneously, the three DWC units supported continuous cultivation of leafy and herbaceous crops, including lettuce (*Lactuca sativa* var. *capitata*), basil (*Ocimum basilicum*), broccoli (*Brassica rapa* subsp. *sylvestris*), and parsley (*Petroselinum crispum*), maintained throughout the production cycle at a density of 25 plants m⁻² under greenhouse natural lighting conditions. Plants were harvested progressively upon reaching commercial maturity and replaced to ensure continuous production.

Water quality parameters, including dissolved oxygen, pH, nitrogen species, oxidation-reduction potential (ORP), and temperature, were continuously monitored using an automated sensor-based system (Senect Control). Fish growth performance and somatic indices were assessed through monthly biometric sampling.

Results

During the nine-month experimental production cycle, the “AWARE” system demonstrated the technical feasibility of aquaponic production fully supplied with reclaimed municipal wastewater. Water quality parameters remained stable throughout the trial ensuring suitable conditions for both fish rearing and plant cultivation. The biofiltration unit effectively supported nitrification processes, allowing the biological conversion of ammonia into nitrite and nitrate while maintaining nitrogen compounds within acceptable thresholds for aquaponic production. No mortality or water quality deterioration was observed during the experimental period.

Nile tilapia exhibited regular growth throughout the production cycle, with progressive increases in body weight and total length and condition factor (K) values indicative of good physiological status (final mean fish weight and length were 418 ± 106 g and 27 ± 3 cm with a K-factor = 2) (**Figure 1**) (FCR = 1.9 and SGR = 0.48). Fish adapted successfully to the reclaimed-water environment, showing normal behaviour and no evidence of stress-related conditions during rearing. The overall growth performance confirmed the suitability of the treated wastewater for sustaining fish production under recirculating aquaponic conditions.

At the end of the production cycle, a total of 55.6 kg of Nile tilapia biomass was harvested. Fish processing generated 20 kg of ready-to-use fillets, corresponding to a fillet yield of approximately 36% of total biomass. The remaining 35.6 kg (64%) consisted of processing by-products, including 7.8 kg of bones, 15.3 kg of heads, and 12.5 kg of viscera. Additional studies are currently ongoing to further evaluate food safety, nutritional composition, and consumer acceptability of the fish products obtained from reclaimed-water aquaponic production.

Simultaneously, the DWC hydroponic units supported continuous cultivation of lettuce, basil, broccoli, and parsley throughout the entire experimental cycle. All crops reached commercial maturity regularly, enabling sequential harvesting and replacement without interruption of production. Plant development was homogeneous among the cultivation units, and no significant nutritional deficiencies or phytotoxic effects were detected, indicating that nutrient availability derived from fish metabolism and microbial mineralization was sufficient to sustain plant growth.

Overall, the results provide the first experimental evidence in Europe that reclaimed municipal wastewater can be successfully integrated into aquaponic food production systems. The “AWARE” system demonstrated stable operation, effective integration between wastewater treatment and biological production, and the capacity to simultaneously support fish and vegetable cultivation within a circular water-food framework. These findings highlight the potential of reclaimed-water aquaponics as an innovative strategy for improving water reuse efficiency and advancing circular economy approaches in European food production systems.

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THE PURSUIT OF NOVEL STRESS MOLECULAR BIOMARKERS: A TRANSCRIPTOMIC APPROACH

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Introduction

Aquaculture has been one of the fastest growing food-production sectors from the last decade. Nevertheless, some barriers still limit the optimization and sustainability of the sector, with stress being one of the main challenges. However, the lack of precise and robust tools to measure stress levels and the effect of mitigation strategies is still a key limitation. Different approaches have been used, such as measurement of physiological indicators, behavioral analysis, and the assessment of molecular biomarkers, which represents a promising approach. However, the currently used molecular biomarkers still present a very high degree of variability and limited predictive power. Therefore, systematic and species-specific evaluation of stress response across multiple stressors is essential to identify robust molecular biomarkers capable of supporting precision monitoring and sustainable aquaculture practices.

Materials and Methods

To address this limitation, transcriptomics analyses were conducted to identify sensitive and precise stress molecular stress biomarkers across three different but highly relevant species for aquaculture production: gilthead seabream (*Sparus aurata*), european seabass (*Dicentrarchus labrax*) and nile tilapia (*Oreochromis niloticus*). Fish were subjected to two different acute stress conditions - ammonia and hypoxia - until a 10% mortality rate was reached (used as a standardized quantitative endpoint of fish stress). Tissue samples were collected at multiple time points after acute stress exposure in order to determine the time window associated with the highest transcriptional response relative to non-stressed controls. Total RNA was extracted from all samples and subjected to high-throughput RNA sequencing. Bioinformatic analyses were then performed to identify differentially expressed genes (DEGs) between stressed and control groups. Candidate biomarkers were selected based on differential expression patterns, functional relevance, and consistency across experimental conditions, resulting in a reduced panel of potential general and stress-specific biomarkers. Finally, selected candidate genes are being validated using quantitative PCR (qPCR) to confirm the RNA-seq results.

Results

Distinct gene expression profiles were observed between control fish and individuals exposed to both stress conditions across all species, confirming that ammonia and hypoxia significantly affected transcriptional responses. Temporal analyses revealed that the magnitude of differential gene expression varied across sampling time points and species. The highest number of DEGs was detected 3 h post-exposure in gilthead seabream, whereas in European seabass and Nile tilapia the peak response occurred at 1 h post-exposure. In gilthead seabream, 21 candidate general stress biomarker were identified, including *fbxo32* and *nr4a3*, along with eight genes ammonia-specific candidates, as *dusp5* and *fzd3*. No hypoxia-specific biomarkers were identified in this species. In European seabass, 10 general stress biomarkers candidates were identified, as *fosh* and *nr4a3*, along with 11 ammonia-specific candidates (e.g. *junbb* and *dusp2*) and two hypoxia-specific candidates, *slc22a7* and *hdac12*. In Nile tilapia, 20 general stress biomarkers (e.g. *avpi1* and *fkbp5*) and 20 ammonia-specific candidates (as *fzd5* and *pold2*) were identified, whereas only four hypoxia-specific biomarkers were detected. Commonly used stress biomarkers, such as heat-shock proteins (*hsp70* and *hsp90*), metallothionein (*mt*) and catalase (*cat*), were also evaluated. Among these, only *hsp*'s showed differential expression, but with lower fold change values when compared to the novel identified biomarkers, and with less consistent responses across stress and developmental stages, as documented in the literature. An exception was observed in Nile tilapia, where *hsp70* ranked among the most strongly regulated general stress biomarkers. Validation in gilthead seabream confirmed the RNA-seq results, with seven general and two ammonia-specific biomarkers maintaining consistent differential expression patterns. Although some genes showed stage-dependent variation, the overall validation supported the reliability of the novel identified biomarkers.

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Discussion

Both general and stress-specific biomarkers were initially identified for each species individually, highlighting species-dependent stress response strategies. Gilthead seabream primarily exhibited metabolic responses, European seabass showed a stronger involvement of signalling pathways, and Nile tilapia exhibited a more integrated response englobing stress signaling, immune and hormonal pathways. Despite these differences, we observed that these different species shared functional response to stress, suggesting the potential for the establishment of cross-species stress biomarkers. For instance, *nr4a3* was consistently differentially expressed across both stress conditions and developmental stages in both gilthead seabass and European seabream. In addition, several gene families were also differentially expressed across species, including FOS, DUSP, F-BOX and TRIM. Notably, a conserved core stress response shared across species was identified, mainly involving three pathways: FoxO, MAPK and Adipocytokine signaling pathways. These findings highlight the potential for the establishment of robust biomarkers for stress monitoring in aquaculture by identifying conserved molecular mechanisms underlying stress responses across multiple species.

Acknowledgment

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FROM AGRICULTURAL EFFLUENTS TO SUSTAINABLE AQUAFEEDS: EVALUATING MICROALGAE EXTRACTS IN PACIFIC WHITELEG SHRIMP (*Penaeus vannamei*) DIETS

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Introduction

The integration of bioremediation into circular economy approaches can provide sustainable sources of novel aquafeed ingredients while contributing to the valorisation of agricultural effluents. In this context, microalgae biomass produced by Necton from agricultural effluents was evaluated as a potential functional feed additive for shrimp post larval rearing. This study investigated the effects of dietary supplementation with *Chlorella* sp. extracts and a combination of *Chlorella* sp. and *Tetrademus* sp. extracts on the growth performance, digestive capacity, tolerance to acute stress, oxidative status, and immune response of *Penaeus vannamei*.

Material and Methods

Five experimental diets were tested in a 26-day feeding trial: a commercial control diet (CTRL) and four experimental diets supplemented with 1% microalgal extracts, including *Chlorella* retentate (ChR), *Chlorella* permeate (ChP), a combination of *Chlorella* retentate and permeate (ChRP), and a mixture of *Chlorella* and *Tetrademus* retentates (MixR). Growth performance, feed conversion ratio (FCR), survival and digestive enzyme activities were evaluated. At the end of the feeding trial, shrimp were subjected to 15-min air-exposure challenge, after which they were returned to their experimental tanks, and mortality was recorded after 1h. The 15-min exposure period was determined in a preliminary trial to achieve approximately 10–20% mortality under the experimental conditions. Oxidative damage (lipid peroxidation; LPO), antioxidant enzyme activities (GPx, SOD and CAT), and stress-related gene expression (*hsp70*, *hsp90*, *ferritin*, *gpx4*, *sod* and *cat*) were analysed in the hepatopancreas. A separate group of shrimp was subjected to a *Vibrio harveyi* immersion challenge for 2 h, after which the animals were transferred to clean seawater and mortality was monitored for 24 h. Following the bacterial challenge, the expression of immune-related genes (*lectin*, *proPO*, *lysozyme*, *alf*, *a2mgl* and *clca1*) was assessed in the gills.

Results and Discussion

Growth performance was comparable between CTRL and ChP, with similar weight gain and FCR, whereas ChR and MixR negatively affected growth. ChRP showed higher FCR compared with other experimental diets. Survival and digestive enzyme activities were not significantly affected by diet. Air-exposure stress resulted in approximately 15% mortality across treatments, with no significant differences among diets. The expression of *hsp70*, *hsp90*, *ferritin*, *sod* and *cat* was not affected by air exposure stress and did not differ among dietary treatments. However, *gpx4* expression decreased after stress in ChR and MixR, while ChR also induced a significant increase in LPO, suggesting an effect in oxidative damage. Antioxidant enzyme activities increased after stress, independently of the dietary treatment. Following *V. harveyi* challenge, mortality ranged from 9% in CTRL to 23% in MixR, with no significant differences among dietary groups. Expression of *lysozyme* was upregulated after infection only in CTRL, whereas *alf* and *a2mgl* expression was increased after bacterial infection in CTRL and ChP. This response was absent in ChR, ChRP and MixR, showing a clear modulation of immune response after a bacterial challenge. Expression of *clca1* was downregulated after infection in CTRL, ChRP and MixR, with no significant response in the remaining diets. Expression of *lectin* and *proPO* expression remained unchanged.

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Overall, the tested microalgal extracts were safe at a 1% inclusion level and did not compromise shrimp survival or digestive capacity. Among the experimental diets, the *Chlorella* permeate (ChP), supported growth and feed efficiency comparable to those of the commercial control diet, whereas the diet combining a mixture of *Chlorella* and *Tetrademus* retentate extracts (MixR) was associated with reduced growth. In addition, *Chlorella* permeate (ChP) influenced immune-related responses under bacterial challenge conditions, suggesting a role in the modulation of immune-related pathways. Although no clear advantages over the control diet were demonstrated, *Chlorella* permeate emerged as the most promising candidate among the tested extracts and warrants further investigation potential as a functional feed additive for *Penaeus vannamei*, particularly through dose-response studies. These findings support the valorisation of effluent-derived microalgal fractions as sustainable feed ingredients within a circular aquaculture framework.

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UNCOVERING GENETIC VARIATION IN PATHOGEN SUSCEPTIBILITY IN SENEGALESE SOLE (*Solea senegalensis*): INSIGHTS FROM BACTERIAL CHALLENGES AND TRANSCRIPTOMIC ANALYSIS

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Introduction

Aquaculture is one of the fastest-growing food production sectors worldwide and plays a crucial role in meeting the increasing demand for sustainable and high-quality protein, with high-value species such as *Senegalese sole* (*Solea senegalensis*) representing important targets for the diversification and economic growth of the industry. The increasing demand for sustainable aquaculture production requires the development of disease-resistant stocks in high-value species such as Senegalese sole (*Solea senegalensis*). However, disease outbreaks still present a major constraint, particularly under intensive farming conditions. Understanding host–pathogen interactions and the genetic basis of disease susceptibility is therefore critical. This study integrates pathogen characterization, experimental infection trials, and transcriptomic analyses to investigate genetic variability in response to bacterial infections in Senegalese sole.

Materials and Methods

Two experimental infection trials will be conducted using *Vibrio scopthalmi* and *Photobacterium damsela* subsp. *piscicida*. Prior to challenge assays, virulence characterization was performed for both bacterial species using specifically designed gene panels targeting known virulence-associated factors. A preliminary challenge (pre-challenge) was conducted for *P. damsela* subsp. *piscicida* to determine the lethal dose 75 (LD75), which was established at 10⁶ CFU. A similar approach will be applied to *V. scopthalmi* to define the appropriate infection dose. In the infection trial with *P. damsela* subsp. *piscicida*, individuals from four genetically distinct families were used to assess differences in susceptibility. A total of 300 fish (mean body weight ~20 g) were distributed across 15 tanks (20 fish per tank). All fish were intraperitoneally injected, with experimental groups receiving 10⁶ CFU of *P. damsela* subsp. *piscicida* and non-infected groups receiving phosphate-buffered saline (PBS). Control fish were maintained in three replicate tanks (20 fish per tank). The challenge had the duration of approximately one week during which mortality was monitored. Tissue samples (intestine, spleen, liver, and kidney) were collected from deceased individuals, survivors and control fish for downstream analyses. Sampling was restricted to freshly deceased individuals to preserve RNA integrity for transcriptomic analyses. To further investigate host response mechanisms, transcriptomic profiling (RNA-Seq) was performed, followed by differential gene expression and pathway enrichment analyses.

Results

The virulence screening revealed the presence of 8 out of 9 targeted genes in *Vibrio scopthalmi* and 9 out of 9 genes in *Photobacterium damsela* subsp. *piscicida*, indicating a high pathogenic potential for both bacterial strains. A preliminary challenge conducted with *P. damsela* subsp. *piscicida* established the LD75 at 10⁶ CFU. Infection trials revealed substantial variability in mortality among families, with cumulative mortality rates of 51%, 89%, 61%, and 95%. Transcriptomic analysis identified differentially expressed genes between experimental groups and highlighted significant enrichment of metabolic pathways associated with the host response to infection.

Discussion

The marked differences in mortality among genetically distinct families suggests a strong genetic component underlying disease susceptibility in *Solea senegalensis*. Such variability, observed under controlled infection conditions, highlights the complexity of host–pathogen interactions and the potential for selective breeding strategies targeting disease resistance. The transcriptomic results suggest that differential regulation of metabolic pathways may play a key role in the host response to infection and contribute to the observed variation in survival between families. These findings reinforce the relevance of integrating phenotypic and molecular data to better understand resistance mechanisms.

This study provides novel insights into host–pathogen interactions and the molecular basis of differential susceptibility to bacterial infection in *Solea senegalensis*, contributing to the identification of candidate markers for selective breeding and improved disease management in aquaculture.

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INTEGRATED EPIGENOMIC DNA METHYLATION AND TRANSCRIPTOMIC LANDSCAPES REVEAL SEX-SPECIFIC LIVER RESPONSES TO CONTINUOUS LIGHT IN ATLANTIC COD (*Gadus morhua*)

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Introduction

The application of continuous light (CL) acts as a potential regulator of delayed puberty in aquaculture Atlantic cod (*Gadus morhua*). However, the epigenetic mechanisms underlying this process have not yet been studied. DNA methylation is an epigenetic modification that enables organisms to respond to stress or environmental changes and regulates gene expression, thereby influencing the phenotype. It is known that gene expression can increase or decrease depending on the genomic location of DNA methylation, and it often causes gene silencing when present in promoter regions. In this context, this study analyzed the transcriptomic and epigenetic profile of Atlantic cod under CL conditions, with the aim of elucidating the molecular and hormonal factors that promote increased growth and delayed sexual maturation.

Materials & Methods

Sixteen-month old Atlantic cod were divided into two groups and exposed to either ambient light (AL) or CL for 6 months. To investigate the interplay between the epigenome and transcriptome, 36 liver samples (18 females and 18 males) were analyzed. Global DNA methylation was mapped at single-cytosine resolution via enzymatic methylation sequencing (EM-seq), with differentially methylated regions (DMRs) and sites (DMCs) identified using the DSS R package. RNA-seq libraries were prepared using the NEBNext Ultra II Directional kit and raw counts were used to identify differentially expressed genes (DEGs) across treatment groups using the R package Limma. To relate DNA methylation patterns to gene expression, genes exhibiting both differential methylation (DMG) and changes in DEG were selected and termed differentially methylated and differentially expressed genes (DEMG). The correlation between DMCs levels and DEG was evaluated using an integration analysis, with the following significance thresholds: DMCs were defined as sites with a minimum methylation difference of 15% and a False Discovery Rate (FDR) < 0.05, and a |Log2 fold change (FC)| ≥ 1 and *P* adj < 0.05 (Benjamini–Hochberg multiple test correction method) for DEGs. The correlations and data distribution were visualized in the R using the ggplot2 package.

Results

Transcriptome analysis identified 1,527 DEGs, in females exposed to CL compared to their counterparts reared under AL. They were enriched in pathways such as “Biosynthesis of unsaturated fatty acids,” “Fatty acid metabolism,” and “PPAR signaling pathway”. In males, 1,788 DEGs were identified, and the metabolic pathways were enriched for “DNA replication” and “ECM-receptor interaction”, suggesting a sex-specific response to photoperiod. Methylation analysis identified a total of 155 DMCs, which were located in the following genomic regions: 88 in promoters, 25 in introns, and 28 in distal regions in females. In contrast, males exhibited a total of 201 DMCs, which were distributed across 76 introns, 68 promoters, and 57 distal regions. A comprehensive analysis of DNA methylation and transcriptome data from Atlantic cod liver identified genes that exhibited differential methylation and expression. In males, the intersection focused on cellular homeostasis and neural support genes, such as *gpx3* (glutathione peroxidase 3) and *smpl* (schwann cell myelin protein-like). Conversely, females exhibited regulation of apoptotic and metabolic signaling pathways, highlighted by *casp6* (caspase-6) and *c1qtnf6b* (C1q and TNF related 6b). These results demonstrate that CL triggers a complex epigenetic-transcriptomic interplay that may reconfigure liver metabolism, providing new insights into the sex-specific response to photoperiod, due to interactions between epigenetic modifications and gene expression.

Acknowledgment

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IN VINO VERITAS – COMBINATION OF PLANT MATERIALS AS A FISH OIL SUBSTITUTE IN DIETS FOR *Salmo salar*

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Introduction

Fish are an important component of human nutrition due to their high dietary value, particularly as a source of the essential long-chain polyunsaturated fatty acids (LC-PUFA) EPA (20:5 n-3) and DHA (22:6 n-3). Since fish cannot synthesise omega-3 fatty acids *de novo*, these must be provided through the diet, with fish oil conventionally serving as the primary source (Tocher, 2015). In the context of sustainability and limited wild fish stocks, alternative lipid sources are gaining increasing attention. However, plant oils do not supply EPA or DHA, leading to declining omega-3 levels in farmed fish. The stilbenoid resveratrol, naturally occurring in grapes, has been shown to increase EPA and DHA levels in rainbow trout when included in the diet (Torno et al., 2017). Because resveratrol can reduce feed intake, lecithin may help counteract this effect by improving palatability (Kaiser et al., 2024; Tocher et al., 2008). Ahiflower oil, rich in stearidonic acid (SDA, 18:4 n-3), offers an additional opportunity to enhance endogenous LC-PUFA biosynthesis. Combining rapeseed oil, resveratrol, lecithin, and ahiflower oil may therefore represent a promising strategy to increase EPA and DHA levels in fish without relying on marine resources.

Materials and Methods

Four diets containing different combinations of rapeseed oil, ahiflower oil, and a lecithin mixture were formulated. Each of these diets was additionally prepared with resveratrol, resulting in eight experimental diets. A fish-oil-based diet served as the control. A 62-day feeding trial with Atlantic salmon was conducted in a RAS facility at Fraunhofer IMTE. Fish were fed twice daily on weekdays and once daily on weekends to apparent satiation.

Results and Discussion

Diets containing rapeseed oil, ahiflower oil, lecithin, or their combinations supported feed intake, feed conversion ratio, and specific growth rate comparable to the fish-oil control. In contrast, the inclusion of resveratrol reduced all growth-related parameters, likely due to decreased feed intake. Ahiflower oil significantly increased total omega-3 fatty acid levels in whole-body fish samples, in line with studies regarding rainbow trout (Fickler et al., 2018). Particularly, fish receiving ahiflower oil showed elevated ALA, SDA, and EPA contents. Interestingly, resveratrol supplementation lowered EPA levels, which may also be linked to reduced feed intake.

Conclusion

Plant-derived lipid sources show strong potential as sustainable alternatives to fish oil in salmon diets. Ahiflower oil effectively increased omega-3 fatty acid levels, including EPA, in Atlantic salmon. The lecithin mixture proved to be a suitable lipid component, supporting growth and maintaining fatty acid profiles comparable to fish oil. Resveratrol, however, appears unsuitable for Atlantic salmon due to its negative effects on palatability and growth performance.

Acknowledgment

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THE ECONOMIC VALUE OF AI AND MACHINE LEARNING BASED OPERATIONS AND MAINTENANCE SUPPORT IN RECIRCULATING AQUACULTURE SYSTEMS: FROM OPERATIONAL ANALYSIS TO IMPLEMENTATION

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Introduction

Economic success in recirculating aquaculture systems (RAS) is not guaranteed. High CAPEX and substantial OPEX for feed, energy, labour and system operation create narrow margins for operational error. Small deviations in feeding efficiency, mortality, or system performance can quickly translate into significant financial losses (Martins et al., 2010; Badiola et al., 2012).

At the same time, RAS generates increasing volumes of operational and biological data. However, value is only created when this data is translated into actionable decisions with measurable economic impact (Føre et al., 2018). In particular, fish behaviour, provides an integrated indicator of feeding response, stress and health (Martins et al., 2012).

The objective of this study is to quantify the economic value of AI-driven operations and maintenance (O&M) support in RAS by defining and evaluating key use cases in RAS and to prioritise their implementation based on financial relevance and technical feasibility.

Material and Methods

Within the Mariculture 2 project at the Rostock Ocean Technology Center, a use-case valuation was developed based on a marine RAS investor business (≈321 T production; farm gate price ~14.5 €/kg).

Four operational categories were analysed: (1) technical system operation; (2) water quality and biological treatment; (3) fish behaviour and feeding; (4) operational competence and knowledge transfer.

Within each category, 3–4 use cases were evaluated against their effect on cost drivers (feed, labour, energy, oxygen, chemicals, CAPEX allocation), and key financial drivers (mortality, feed efficiency, labour effort and system downtime). Measurable signals were linked to operational interventions and translated into annual economic impact (€ per production unit).

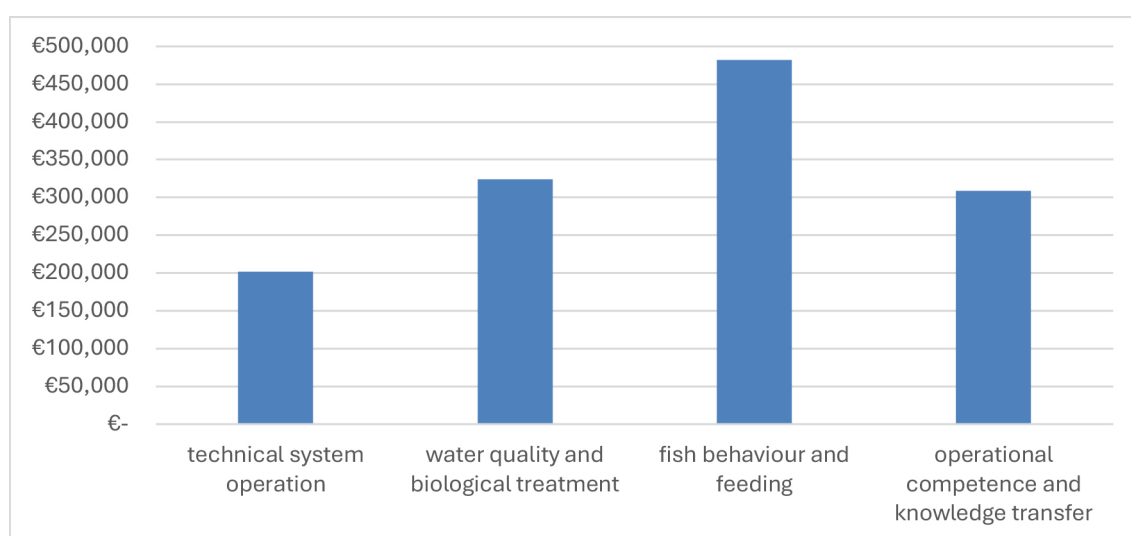


Fig. 1., Economical potential of the four use-case categories evaluated.

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Results

Total economic potential for the application of AI and machine learning based O&M support was estimated at ~1.3 M€/year per production unit (Fig. 1).

Fish behaviour and feeding showed the highest impact (~482 k€/year), driven by feeding optimisation and early detection of stress and health issues. Water quality management contributed ~324 k€/year through stabilised production conditions. Competence and knowledge transfer reached ~309 k€/year via improved decision-making and standardisation. Technical system optimisation showed lower value (~202 k€/year) but higher implementation readiness.

Discussion

Highest value is generated where data directly focus on the fish as object of interest. Fish behaviour and specifically group behaviour integrates feeding, water quality and health, making it a key leverage point but also technically demanding.

To address group behaviour evaluation, a pre-project will validate camera-based behavioural monitoring of fish groups in large-scale RAS environments. In parallel, an implementation roadmap towards a minimum viable product (MVP) is developed, defining IT/OT architecture, data integration and decision-support tools to roll out RAS O&M support.

All activities targeting towards the implementation of the AI driven O&M support to be implemented with the first floating RAS, planned to be operational in 2027.

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ONE HEALTH SURVEILLANCE OF ANTIMICROBIAL RESISTANCE AND HEAVY METAL CONTAMINATION IN CLARIID CATFISH AQUACULTURE AND POST-HARVEST SYSTEMS

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Introduction

Catfish aquaculture plays an increasingly important role in food security, rural livelihoods, and value-added fish processing in Southeast Asia. However, intensification of catfish production has raised One Health concerns due to the combined emergence of antimicrobial-resistant bacteria and environmental contaminants, particularly heavy metals, which may affect aquatic animal health, food safety, consumer health, and surrounding ecosystems. In Thailand, clariid catfish are widely consumed and are also processed into fermented fish products that support local economies. Nevertheless, aquaculture water, fish tissues, and post-harvest processing chains may serve as reservoirs and transmission interfaces for pathogenic bacteria, antimicrobial resistance (AMR), virulence factors, and chemical contaminants. This study presents an integrated One Health framework to evaluate antimicrobial resistance, virulence-associated bacteria, and heavy metal-related risks in clariid catfish aquaculture and fermented fish production systems.

Materials and Methods

Fish and water samples associated with clariid catfish aquaculture and fermented catfish production were collected from production areas in Thailand. Bacterial isolation was performed using selective culture media targeting *Escherichia coli*, *Salmonella* spp., and *Vibrio cholerae*. Antimicrobial susceptibility testing was conducted using the disk diffusion method against nine antibiotics, and multidrug resistance profiles were assessed. Polymerase chain reaction (PCR) was used to detect selected antimicrobial resistance genes and virulence genes. To broaden the One Health interpretation, available evidence on heavy metal contamination in hybrid catfish aquaculture systems was integrated with environmental risk concepts, including water quality, tissue bioaccumulation, oxidative stress biomarkers, transcriptional responses, and human health risk assessment.

Results

Bacterial isolates, including *E. coli*, *Salmonella* spp., and *V. cholerae*, were detected in clariid catfish and water samples associated with fermented fish production. Antimicrobial susceptibility testing revealed diverse resistance patterns, with multidrug resistance observed particularly among *E. coli* and *Salmonella* spp. The resistance profiles were mainly associated with β -lactam and tetracycline antibiotics, suggesting potential selective pressure from antimicrobial use and environmental contamination. Molecular assays confirmed the presence of antimicrobial resistance genes and virulence-associated genes among bacterial isolates, suggesting that catfish production environments may function as reservoirs of foodborne and antimicrobial-resistant bacteria. In parallel, evidence from heavy metal risk assessment in catfish aquaculture demonstrated that canal-specific environmental contamination can drive metal bioaccumulation, oxidative stress, altered expression of detoxification and immune-related genes, and elevated non-carcinogenic and carcinogenic health risks at contamination hotspots.

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Discussion

These findings support the view that AMR and heavy metal contamination should not be treated as separate problems in aquaculture, but rather as interconnected One Health pressures linking aquatic environments, farm management, fish health, food processing, and consumer safety. The detection of antimicrobial-resistant and potentially virulent bacteria in clariid catfish and water used for fermented fish production highlights the need for continuous microbiological surveillance, responsible antimicrobial use, and improved biosecurity. At the same time, heavy metal bioaccumulation and molecular stress responses emphasize the importance of environmental monitoring beyond farm-level certification, particularly where aquaculture systems rely on canal networks influenced by urban, agricultural, or industrial land use. An integrated One Health surveillance platform combining AMR monitoring, virulence gene detection, heavy metal assessment, and environmental risk mapping would strengthen food safety management and support the sustainable development of catfish aquaculture and fermented fish value chains.

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HYDROLYSATE-ENRICHED FISHMEAL SUPPORTS PERFORMANCE IN ATLANTIC SALMON *Salmo salar* L. FOLLOWING THERMAL LICE TREATMENT

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Introduction

Acute delousing stress in Atlantic salmon (*Salmo salar* L.) significantly reduces post-treatment feed intake, impairing growth, health and welfare (Bowers et al., 2000; Grave et al., 2004; Hamre et al., 2009; Igboeli et al., 2014). Identifying factors, including dietary ingredients and bioactive components, that can mitigate these stress effects and help fish resume feeding sooner is therefore essential. The side-stream biomass from fish catch, in the form of abundant and nutrient-rich fish protein concentrate (FPC), represents an underutilized resource with potential for valorization, enhancing sustainability by reducing future aquaculture’s dependency on fish meal. However, not all feed factories are equipped to utilize wet silage products, hence enriching fishmeal (FM) with added hydrolysate makes it a more suitable ingredient.

Materials and Methods

This study evaluated the effectiveness of dietary FPC and two enriched FMs, either with FPC (FPCM), or with fish protein hydrolysate (FPHM), compared to a commercial FM, in mitigating stress from exposure to repeated simulated delousing treatments in salmon (428 to 894 g body weight). Five diets were tested: low fishmeal (LFM; 7.5% FM); medium fishmeal (MFM; 15% FM); FPC (7.5% FPC + 7.5% FM) and two hydrolysate-enriched fishmeal diets (7.5% FPCM + 7.5% FM and FPHM + 7.5% FM). Salmon performance was evaluated under optimal rearing conditions for 3 months, interrupted by three acute heat (32 °C; 30 sec) and crowding (15 min) stress treatments mimicking a commercial acute high temperature delousing (AHTD) treatment (Grøntvedt et al., 2015; Noble et al., 2018) with three weeks between each treatment.

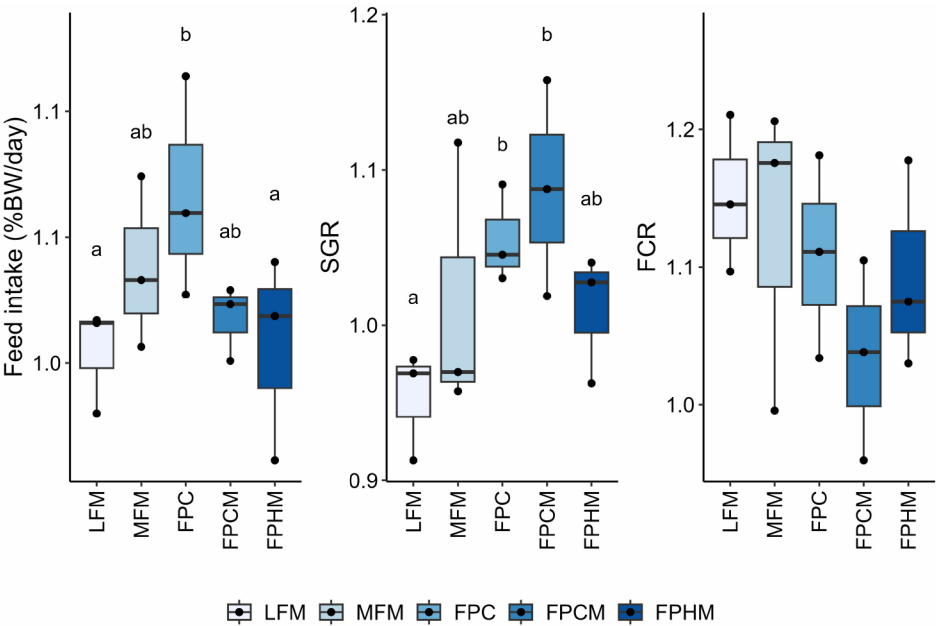


Figure 1. Feed intake (%BW/day) (left), SGR (mid) and feed conversion rate (FCR) (right) in fish fed LFM, MFM, FPC and hydrolysate-enriched fishmeals FPCM & FPHM during repeated episodes of acute heat lice treatment stress (N=3). One-way, ANOVA P < 0.05.

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Results and discussion

By the end of the trial, fish fed the diets containing FPC or the enriched FMs showed similar feed intake and growth compared to the MFM treatment, while the FPC had higher feed intake compared to LFM and FPHM, and FPC and FPCM treatments resulted in higher growth compared to LFM. Fish plasma cortisol, glucose, and lactate levels were elevated after repeated AHTD treatments, confirming a sustained stress response. Dietary effects on plasma metabolites and qPCR analyses for genes involved in the gut-brain axis control of appetite and energy utilization in salmon will be presented. In conclusion, diets with low-impact and circular ingredients, such as fish protein concentrate (FPC) and a fish meal product enriched with fish protein concentrate (FPCM), can support similar feed intake and performance as high-quality fishmeal during periods of repeated acute stress. FPC contributed to sustained high feed intake, while FPCM gave higher efficiency, following repeated occurrences of acute heat stress treatments simulating commercial heat treatments against lice.

Acknowledgment

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DEVELOPING NURSERY CULTURE PROTOCOLS FOR GREENSHELL™ MUSSEL (*Perna canaliculus*) SPAT: LINKING DIETARY POOLS, FLUX, AND BIOMASS DEMAND

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Background

Greenshell™ mussels (*Perna canaliculus*) underpin New Zealand's aquaculture sector, yet production remains highly inefficient. Nearly 400 billion spat are harvested annually to yield only 1.5 billion harvestable mussels (Skelton et al., 2022). A major driver of this inefficiency is the extremely small size of spat used for seeding, typically just 0.5 to 1 mm in shell length, which makes them highly vulnerable to post-seeding losses. Rearing spat to larger sizes in nursery systems could greatly improve retention, reduce spat wastage, and increase farm productivity. The first step to developing scalable, predictive nursery systems is to determine the relative importance of different dietary pools to spat growth. This would enable nursery managers to monitor food availability and better predict nursery performance. In this study, spat were reared in an upwelling nursery system to quantify the relative depletion of phytoplankton, dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and particulate organic matter (POM), providing an initial assessment of the relative importance of different dietary pools.

Methods

Spat were reared in a land-based upwelling nursery system supplied with raw seawater from an adjacent bay in the Marlborough Sounds. Water samples were collected from the system inflow and outflow to quantify changes in phytoplankton, DOC, DON, and POM.

Results and Discussion

Phytoplankton showed the greatest and most consistent depletion across sampling periods (peaking at 21.1% of the available pool), indicating it was the primary dietary resource for spat. POM was depleted to a lesser extent, while DOC and DON showed low or inconsistent depletion, suggesting a comparatively minor nutritional role. This study provides an initial step toward identifying the key dietary pools supporting Greenshell™ mussel spat in nursery systems. These results identify the dietary pools most likely to influence nursery performance. Future work will quantify the assimilation efficiency and growth value of each pool, enabling the development of biologically meaningful food metrics for predictive nursery management.

Acknowledgments

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GROWTH AND MATURATION IN ATLANTIC SALMON POST-SMOLT: PRODUCTION-RELEVANT ROLES OF PHOTOPERIOD, DIET AND LIPID DEPOSITION

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Background and aim of the experiment

Photoperiod and the direction of changes in daylength play a central role in regulating puberty, but their effects seem closely linked to energy status and whole-body fat accumulation, indicating a potential interaction between environmental cues and internal energy reserves. In aquaculture production of Atlantic salmon, early sexual maturation is an undesirable developmental process that ultimately leads to reduced growth performance and quality downgrading. This challenge is especially common in males reared in closed containment systems such as post-smolt facilities, where continuous light, elevated water temperatures, and high-energy diets are applied to promote rapid growth. However, these factors may also accelerate the onset of puberty. Therefore, identifying production regimes that limit early sexual maturation without compromising growth performance requires further investigation and research. In this study, Atlantic salmon post-smolts reared in brackish water (20ppt) growing from 100 - 800g, were divided into four groups which were exposed to continuous (1), increasing (2) and decreasing (3) daylength, all fed a standard protein-to-lipid ratio, and one group exposed to decreasing daylength was fed a high dietary protein-to-lipid ratio (4). All fish were then transferred to seawater (800 - 2400 g) and exposed to decreasing daylength to assess the effects of the treatments on key aspects of salmon production, including energy allocation, growth performance, and sexual maturation. Growth performance and sexual maturation were assessed through repeated measurements of individual fish at eight time points, combined with biometric measurements, energy status indicators, hormone profiling, and macroscopic and histological examination of the gonads.

Abbreviations and clarifications:

11-KT: 11-ketotestosterone

GSI: gonadosomatic index = gonad weight / live body weight x 100

VSI: viscerosomatic index = visceral package (visceral fat and intestines) / live body weight x 100

Results and discussion

The initial photoperiodic treatments significantly influenced male maturation rates and growth performance. Exposure to continuous light (1) led to relatively high male maturation rates (29.4%) while maintaining high growth performance (TGC = 3.62). Salmon exposed to increasing daylight (2) exhibited high male maturation rates (44.4%) and lower growth performance (TGC = 3.48), while gradually decreasing daylength (3) exposure reduced the incidence of male maturity (4.3%) without compromising growth performance (TGC = 3.69). The high protein-to-lipid ratio (4) treatment significantly reduced lipid deposition and visceral weight, which in turn affected the body composition and suppressed testicular development. However, reduced lipid deposition did not translate into improved overall growth performance (TGC = 3.69) after seawater exposure or reduced maturation rates (3.8%).

Furthermore, when the fish had reached a body weight of approximately 800g, before seawater exposure, males were thoroughly analyzed for signs of sexual maturation. Based on serum 11-KT levels and GSI a significant positive correlation ($r = 0.52$) was observed between 11-KT and VSI among immature males (GSI <0.05, 11-KT <2ng/ml). A similar positive correlation ($r = 0.80$) was found for pubertal males (GSI <0.05, 11-KT >2ng/ml), whereas a non-significant negative correlation ($r = -0.60$) was observed between these traits for maturing individuals (GSI >0.05, 11-KT >2ng/ml). This implies that visceral fat is an important energy storage site related to pubertal development.

Besides the contribution into insight on the role of visceral fat in pubertal development, these results provide valuable knowledge for the development of more effective production protocols of post-smolt Atlantic salmon in closed containment systems regarding photoperiodic exposure, and dietary strategies balancing lipid deposition, to prevent early sexual maturation without compromising growth performance.

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The abstract is based on findings from the following two published studies as well as additional unpublished data.

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e-CITIZEN SCIENCE COMMUNICATING: ARTIFICIAL INTELLIGENCE FEEDBACK BETWEEN OBSERVATIONS AND STAKEHOLDER INPUTS FOR CROSS-BORDER RESILIENT FISH MIGRATION IN EUROPE

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Introduction

Migratory fishes connect rivers, estuaries and seas while crossing administrative and policy boundaries; however, European management remains fragmented and feedback between emerging science, local observations and operational decisions is often weak. HORIZON Project FISH2SUSTAIN addresses this challenge through life-cycle, multi-stressor research and bioclimatic modelling (Nekrasova et al. 2024) on three model migration strategies: long-distance catadromous European eel (*Anguilla anguilla*), river-sea-river anadromous Atlantic salmon (*Salmo salar*), and coastal-marine European seabass (*Dicentrarchus labrax*). Citizen science plays an important role in ensuring sustainable nature conservation (Bonney et al. 2014) and adaptive feedback between observations and stakeholder inputs for transboundary sustainable fish migration in Europe.

Materials and Methods

The Project FISH2SUSTAIN develops a stakeholder-centric machine-learning e-Citizen Science environment (Figure 1) based on mobile application with AI-Stakeholder co-creation and interaction, targeted e-Education, anonymous One-Click geolocated photo-reporting, real-time data collection and knowledge gamified testing for different stakeholder MARG (Pupins et al. 2025). The present study aims to design and validate this e-Citizen Science AI-component as a bidirectional, evidence-aware interface linking citizen/stakeholder observations with the validated scientific outputs of the whole project and returning interpretable, uncertainty-aware adaptive feedback.

Results

As this research is implemented within the FISH2SUSTAIN work plan, results are formulated as expected and testable outputs rather than completed findings. The primary result will be an operational e-Citizen Science mobile application that shortens the pathway from a local observation of a migrating fish to a quality-controlled, spatially explicit and FAIR-compatible record. The AI assistant will make complex project evidence accessible without presenting predictions as observations or suppressing uncertainty. Validated citizen records will provide an additional stream for detecting migration events, spatial gaps, bottlenecks and unusual environmental conditions and will feed the adaptive feedback architecture. Performance will be quantified against expert-labelled observations and independent monitoring, while AI-stakeholder co-creation will test whether feedback improves understanding, reporting quality and sustained e-Engagement. The target architecture is a closed observation-to-action loop (Figure 1), in which user inputs improve monitoring and model evaluation and validated Project knowledge is returned to users in accessible form.

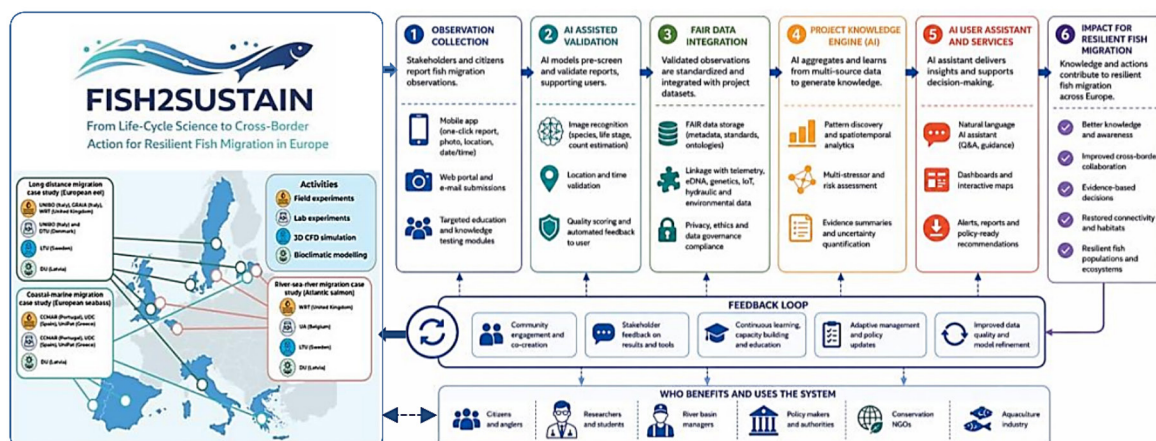


Figure 1. FISH2SUSTAIN stakeholder-centric machine-learning e-Citizen Science environment and AI Pipeline Loop.

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Discussion

The proposed system extends e-Citizen Science from one-way data acquisition to continuous science-policy-society co-investigation. Its novelty lies in coupling low-burden geolocated reporting with expert-governed AI, project-wide mechanistic evidence, bioclimatic modelling, and a Digital Twin. This design can strengthen cross-border comparability and enable local observations for sustainable cross-border management (Pupins et al. 2026).

Acknowledgment

This research is developed within the Horizon Europe FISH2SUSTAIN project “From Life-Cycle Science to Cross-Border Action for Resilient Fish Migration in Europe”. We thank INTERREG project UrbUmbrella, Nature Conservation Agency for the cooperation.

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CATEGORIZING SOLUTIONS FOR SLUDGE COLLECTION AND PROCESSING ON SITE FOR FLOATING CLOSED CONTAINMENT SYSTEMS

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Introduction

Floating closed containment systems (FCCS) are increasingly being implemented in Norwegian Atlantic salmon aquaculture due to regulatory constraints that limit further growth, such as regulations related to salmon lice. Fish farmers are also required to conduct environmental monitoring of the seabed beneath and surrounding aquaculture sites. FCCSs are physically enclosed with designated inlets and outlets allowing for control over emissions to the environment, which often includes sludge collection.

Solutions for sludge collection and processing vary in design and operation, due to differences in fish farmers' objectives and contexts. Sludge collection and processing covers three steps on site; (i) collection of sludge at, often designated, outlets within the production unit, (ii) transport of sludge from production unit to processing unit, (iii) increasing the dry weight percentage of the sludge at the processing unit.

This work covers mapping and categorization of current solutions for sludge collection and on-site processing for FCCSs. First, all FCCSs and relevant sludge processing systems commercially available in Norway are identified and listed in an overview. Then, relevant information on these solutions is collected through document study, interviews and field visits. This covers information on working principles, collection rate, energy consumption, safety for workers, reliability, space/size, capacity, operational limitations (motions, heeling, etc.), and more. Finally, the solutions are assessed and organized into categories emphasizing how they are suited to serve different purposes.

Results and discussion

A total of 26 different FCCS systems have been identified in operation or being sold to customer and currently under construction. The level of sludge collection and treatment differs significantly both as a result of technological limitations and preferences on operation – that is, how much the fish farmer wants to collect. Some concepts have been in operation for almost ten years, while other concepts have only recently been sold or are updated versions of older concepts.

Work on collecting information and assessing the solutions for sludge collection are currently in process, and results will be ready in time for Aquaculture Europe 2026.

Acknowledgment

This research is part of the ongoing project “Solutions for sludge collection from closed containment systems at sea and their implications for the external environment”, financed by the Norwegian Seafood Research Fund (project number 910671).

ESTIMATES ON SLUDGE PRODUCTION AND COLLECTION RATES FOR OPEN SEA-BASED AQUACULTURE OF ATLANTIC SALMON (*Salmo salar*)

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Introduction

Environmental impact from sea-based finfish aquaculture is becoming increasingly prioritized in the authorities handling of the industry. In Norway, this has led to requirements for collection of sludge from Atlantic salmon aquaculture sites in addition to the regulations on the near-field effects on the sea bed. In addition, sludge from aquaculture is increasingly viewed as a resource towards other products such as fertilizers, feed and energy. However, the amount and quality of sludge being produced and available for collection from sea-based aquaculture is not sufficiently investigated. In this work state of the art on mass-balance calculations for feces production for Atlantic salmon, and properties of feces and feed spill is reviewed before drift of feces and feed spill is analysed. Thereby, collectable proportion of dry weight is estimated.

Methodology

A literature review based on a structured search mixing relevant keywords such as sludge, collection, aquaculture, marine, feces, feed spill, sinking/sinking velocity, mass balance, salmon, waste, pellets, current, dispersal, digestibility, and more was performed. 62 scientific papers and reports were found to be relevant for Atlantic salmon fish farming, and were divided into three categories: (i) Mass balance, (ii) Feces and feed properties, and (iii) Collection of feces and feed. Quality of experiments, consensus between researchers and publications, and knowledge gaps were assessed to understand the status of current knowledge.

Based on knowledge from the literature review, analyses on feces and feed spill drift in sea were performed. Understanding how feces and feed spill travel after they are released into the sea in the net pen is crucial to estimate collection rates for sludge collection systems. Two approaches were combined; First a limited number of analyses using Computational Fluid Dynamics (CFD) were performed for variations in drop points, densities, drag coefficients, settling velocities and current speeds. Then, a simplified model was tuned to match the results of the CFD-analyses as closely as possible. A series of scenarios for aquaculture production and environmental conditions were then assessed using the simplified model.

Results and discussion

Mass balance is an established concept for quantifying inputs and outputs in aquaculture systems and is frequently extended to include feed waste. This approach enables the establishment of critical relationships between feed input, fish growth, and production contributions. For Atlantic salmon, mass balance formulas are generally based on the quantity of feed consumed, the amount digested, and the nutrients retained within the fish. Consequently, feed that remains unconsumed is classified as feed spill, undigested material as feces, and non-retained nutrients as excretion.

While the literature provides numerous parameter values for these calculations, they are often inconsistent, case-specific, and difficult to validate for novel applications. This poses a significant challenge for accurate estimation and strategic planning. Digestibility is inherently dependent on feed composition and varies across different nutritional components, with some studies also indicating variations linked to fish size. Furthermore, the effect of environmental factors such as temperature introduce additional complexity to these estimates.

Fecal composition depends on feed ingredients and fish digestion, though it does not appear to exhibit substantial variation. In contrast, fecal shape, size, consistency, and settling velocity appear to vary more significantly depending on fish size, feed types, and the potential inclusion of binders. Fecal content typically remains within defined intervals, with settling velocities falling within these specific ranges.

Simulations of particle dispersion and the capture efficiency of a collection funnel were therefore conducted using various parameter settings, including different sizes, drag coefficients, and weights. Particles were released from multiple areas within the cage to represent the spatial distribution of a fish population. A collection funnel was placed in two different positions beneath the cage, and the percentage captured was measured across varying current velocities.

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The CFD analysis demonstrates that results range from 0.0% to 97.4% capture efficiency within reasonable variations in the experimental setup. The simplified model demonstrated a strong capability for adaptation and replication of the CFD results by adjusting the flow velocity reduction through the net wall. However, an inherent inaccuracy remains, as the model produces both higher and lower values than the CFD results for different parameter choices at the same reduction factor. This is likely due to the uniform flow field in the simplified model, whereas the CFD analysis accounts for significant spatial variation—specifically, that current velocity is higher beneath the cage than inside, and that it decreases toward the aft of the cage.

Combining these results allows estimating collectable emissions for concrete setups for sludge collection. This gives valuable insight into the true efficiency of mitigation measures for particulate emissions and environmental impact from fish farms.

Acknowledgment

This research is part of the ongoing project “Calculation model for emission and collection of sludge from fish farms at sea”, financed by the Norwegian Seafood Research Fund (project number 910382).

EFFECT OF A NEXTGENERATION PHYTASE ON PERFORMANCE, MINERAL UTILISATION AND MYOINOSITOL METABOLISM IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

The increasing inclusion of plantbased raw materials in aquafeeds reduces phosphorus (P) availability due to phytate, increasing dependency on inorganic P supplementation and contributing to environmental P discharge. Exogenous phytase is widely used to improve phytatebound phosphorus utilisation in fish. Beyond phosphorus release, phytase supplementation has been associated in terrestrial species with so-called extraphosphoric effects, potentially mediated by myoinositol liberation from phytate. Evidence for such mechanisms in salmonids, however, remains limited. This study evaluated the effects of a nextgeneration phytase on growth performance, nutrient digestibility, mineral status and myoinositol metabolism in rainbow trout.

Materials and Methods

An 84day feeding trial was conducted using rainbow trout (*Oncorhynchus mykiss*; initial body weight 69.5 g) housed in a recirculating aquaculture system. Fish were fed a plantproteinrich, moderately phosphorusdeficient diet (negative control; total phosphorus 0.65%) or a positive control diet supplemented with inorganic phosphorus (total phosphorus 0.87%). The negative control diet was supplemented with a nextgeneration phytase at 1,000 or 3,000 phytase units kg⁻¹. Each treatment consisted of three replicate tanks with 20 fish per tank. Growth performance, feed conversion ratio, apparent digestibility coefficients of phosphorus and zinc, plasma phosphorus and myoinositol concentrations, and mineral content of vertebrae and scales were determined at the end of the trial.

Results and Discussion

Phytase supplementation significantly improved feed conversion ratio compared with the negative control, reaching values comparable to the positive control. Apparent phosphorus digestibility increased significantly at both phytase inclusion levels, while zinc digestibility improved at the higher dose. Plasma phosphorus concentrations were restored to levels similar to those observed with inorganic phosphorus supplementation. Notably, phytase inclusion resulted in a marked and dosedependent increase in plasma myoinositol concentrations. Enhanced mineral deposition was observed in both vertebrae and scales, with scale mineral content closely reflecting vertebral mineralisation, supporting its use as a practical indicator of skeletal mineral status.

Conclusions

Supplementation of a moderately phosphorusdeficient trout diet with a nextgeneration phytase improved phosphorus utilisation, mineral status and feed efficiency, enabling a reduction in inorganic phosphorus supplementation. The pronounced increase in plasma myoinositol indicates that extraphosphoric effects of phytase are relevant in rainbow trout, extending observations previously described in terrestrial species. These findings support phytase supplementation as an effective nutritional strategy to improve performance and sustainability of plantbased salmonid feeds.

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EFFECTS OF STOCKING DENSITY ON SHRIMP (*Penaeus vannamei*) PERFORMANCE AND HALOPHYTE PRODUCTION IN A COUPLED AQUAPONIC SYSTEM

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INTRODUCTION

Aquaponics is a sustainable food production system that integrates aquaculture and hydroponics, enabling the simultaneous production of aquatic animals and plants while reducing environmental impact. However, limited information is available on the role of stocking density in marine aquaponic systems, particularly regarding its effects on shrimp performance and halophyte productivity. This study aimed to evaluate the effects of different stocking densities on survival, growth performance, product quality of whiteleg shrimp (*Penaeus vannamei*), and biomass production of *Salicornia* in a coupled aquaponic system.

METHODS

The experiment was conducted in a greenhouse aquaponic facility at the University of Padua using a completely randomized design with three stocking densities: low (LD: 65 shrimp/m²), medium (MD: 123 shrimp/m²), and high (HD: 182 shrimp/m²), each with three replicates. Shrimp were reared in recirculating aquaponic systems integrated with vertical *Salicornia* cultivation using nutrient film technique (NFT). The trial lasted 50 days. Water quality parameters (temperature, dissolved oxygen, pH, nitrogen compounds, turbidity, chlorophyll) were regularly monitored. Growth performance (final body weight, specific growth rate, feed conversion ratio, survival), shrimp quality traits (morphometrics, lesion incidence, texture, color, pH), and plant biomass were measured. Statistical analyses included general linear models, logistic regression, and survival analysis.

RESULTS & DISCUSSION

Water quality remained within optimal ranges across all treatments. Turbidity ($P = 0.049$) and chlorophyll ($P = 0.011$) increased with stocking density, indicating higher suspended solids and phytoplankton biomass at higher densities. Survival averaged 85% and was not affected by treatment. Growth performance differed significantly ($P < 0.001$), with shrimp at low density reaching the highest body weight (~13–14 g) compared to medium (~10 g) and high density (~8 g). Conversely, total biomass increased with density ($P = 0.010$), reaching 559.6 g (LD), 783.4 g (MD), and 930.7 g (HD), with biomass growth also higher in MD and HD ($P = 0.014$). Feed conversion ratio was unaffected ($P = 0.823$). Lesion incidence did not differ among treatments but increased with body weight ($P < 0.001$). Shrimp at low density showed higher carcass weight and body size ($P < 0.001$), while most meat quality traits were unchanged; however, cooked redness (a^*) was higher at lower densities ($P = 0.011$). *Salicornia* yield increased with stocking density, reaching 12.05, 10.49, and 9.87 kg m⁻² in HD, MD, and LD, respectively.

Overall, a clear trade-off emerged: lower densities improved shrimp growth and quality, whereas higher densities maximized total biomass and plant production

PHYSIOLOGICAL CHANGES OF NEW REDUCED-FISHMEAL ORGANIC DIET IN TWO GILTHEAD SEA BREAM (*Sparus aurata*) GENETIC LINES

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Introduction

Organic aquaculture, an alternative to conventional aquaculture, requires feeds that meet strict standards for either fish quality, animal welfare and regulatory compliance. The current scenario shows a restrictive expansion due to scarce availability of certified feed ingredients, the ban on using synthetic amino acids, and strict organic rules. Therefore not only shall alternative ingredients be explored but also new genotyped / phenotyped cultured animals. The aim of this study was to evaluate the effects of conventional and organic diets, including a novel formulation with hydrolysed yeast extract and organic poultry meal, on two genetically distinct gilthead sea bream (*Sparus aurata*) lines differing in growth potential.

Materials and methods

Here, two Gilthead sea bream (*Sparus aurata*) genetic lines (fast-growing and standard) coming from Cromaris (Croatia) were fed with different organic and conventional diets (designed by INRAE, Legouessant Aquaculture and Lallemand SAS). One novel organic diet featuring partial fishmeal replacement with hydrolysed yeast extract and inclusion of organic poultry meal, while the other organic diet was a commercial one without yeast extract. Three isoproteic (43%) and isolipidic (17%) diets (conventional (CC), commercial organic (CO), novel organic (NO)) were tested in a 3 × 2 factorial design across eighteen 800-L tanks in a controlled RAS. Fish (133.5 ± 0.25 g) were PIT-tagged, reared under optimal conditions over 94 days. At the end of the trial samples from epaxial white muscle, liver and bone were collected. Texture and colour of muscle were analysed.

Results and discussion

Clear differences were seen between diets and genetic lines, being the fast-growht the weightenest one. Samples from muscle, liver and bone were processed in order to shed light into the differences between genetic lines in regard to their transcriptome, as well as texture and colour of the fillet. Despite observing differences because of genetic factor, fish fed with novel organic diet showed a higher expression on muscle proteolytic genes such as *trim63* and *fbxo32*, and an upregulation in *myod1* (first steps of myogenic development) suggesting differences in muscle remodelling (Figure 1). Novel diet produced a slightly lowest firmness in comparison with the other two diets, as well as a significant colour change. Together with assessing the GH/Igf pathway and the upcoming results of bone and liver expression will help to understand the response of these two genetic lines to organic diets.

Acknowledgement

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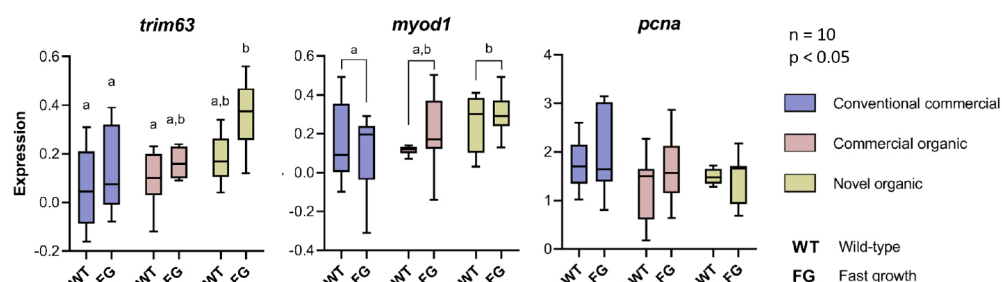


Figure 1. Gene expression in epaxial white muscle of *trim63* (proteolytic), *myod1* (myogenic) and *pcna* (proliferative) in each experimental diet (Post-Hoc test, n = 10, p-value < 0.05).

EFFECTS OF EARLY-LIFE RAS REARING, GENETICS AND DIETARY FAT LEVELS ON PERFORMANCE AND HEALTH OF *A. Salmon* BEFORE AND AFTER SEA TRANSFER

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Background

Mortality in sea of Norwegian salmon production is 14% ([Fiskehelserapporten 2025 - Veterinærinstituttet](#)), whereas the Norwegian authorities aim for 5%. One way to reach this goal is to ensure that the smolt that are set out in the sea are of high quality. The aim of this study was to estimate the effects of early-life RAS rearing, genetics, and dietary fat levels on performance and health of *A. salmon* before and after sea transfer.

Material and Methods

A feed trial with triplicate RAS tanks with two diets (six tanks in total) was performed at the Nofima Research Station for Aquaculture. Fish (ca 1700 fish in total) came from 70 families from Benchmark Genetics including two extreme groups with high (HIGH) and low (LOW) breeding value for fillet fat. Two feeds with different fat levels were produced at Skretting's feed technology plant (Skretting Aquaculture Innovation, Stavanger, Norway). At size 150g, records were obtained for survival, growth, length, condition factor (CF) and external welfare on all fish (including cataract recorded using visual inspection). From 30 fish per extreme groups, hepatosomatic index (HSI) and visceral somatic index (VSI), nephrocalcinosis (0/1) were also recorded. The fish were later transferred to sea cages and fed one feed until slaughter at 750g. The same traits will then be recorded plus fat content of liver and NQC and visceral somatic index. A second sample from the extreme groups were recorded as above at this life stage. All fish were genotyped using an array of Benchmark Genetics containing 70k SNP markers.

Results and Discussion

Before sea transfer fish show significantly higher HSI for the extreme HIGH genetic group compared to the LOW genetic group (1.52 vs 1.36%) and significantly higher HSI in fish fed the highest dietary lipid level (1.51 vs 1.37%). The interaction between genetic group and feed spanned from 1.27 to 1.6%. For VSI, these figures were 8.0 and 7.6% for the genetic group effects and 8.0 and 7.6% for the feed. The interaction between genetic groups and feeds spanned from 7.3 to 8.2g. There was overall high incidence of skin lesions (13.9%), opercular shortening (6.0%) and cataract (55%). There were small differences in production traits between the feeds (Feed Conversion Ratio (0.75 and 0.77, $p=0.07$) for high and low fat diets, Specific Growth Rate (1.41 and 1.37, $p=0.05$) and Thermal Growth Coefficient (2.00 and 2.07, $p=0.07$). Final results come in May.

Acknowledgment

This project has received funding from the European Union's Horizon Europe Research and Innovation Actions programme under grant agreement N° 101181159.

EVALUATION OF *Schizochytrium* OIL AS A FISH OIL REPLACEMENT IN DIETS FOR ATLANTIC SALMON *Salmo salar*

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Introduction

Fish oil (FO) has been used as a major lipid source in aquafeeds due to its abundant long-chain n-3 polyunsaturated fatty acids (LC-PUFA), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are essential for growth and fillet quality of carnivorous marine fish such as Atlantic salmon (*Salmo salar*). However, the increasing global demand and limited supply of marine-derived oils have raised concerns regarding the long-term sustainability and economic stability of FO. As an alternatives lipid sources, oils derived from heterotrophic microalgae such as *Schizochytrium* have received considerable attention. *Schizochytrium* oil is particularly rich in DHA and can be produced independently of marine fisheries, making it a sustainable and scalable source of n-3 LC-PUFA. Dietary lipid composition plays a critical role in regulating membrane structure, metabolic processes and overall physiological performance in fish. These physiological functions are closely associated with the ability of fish cope with environmental stress. In particular elevated water temperature has become an increasingly important challenge in Atlantic salmon aquaculture due to climate change and seasonal warming. Heat stress can impair feed intake, growth performance, oxidative balance, immune competence and physiological condition of Atlantic salmon. Membrane lipid composition and antioxidant capacity are closely associated with thermal tolerance, suggesting that diets rich in DHA and other bioactive lipids may improve resilience under high-temperature conditions. *Schizochytrium* oil contains high concentrations of DHA and antioxidant compounds such as carotenoids. Therefore, it may provide functional benefits beyond functioning as a conventional FO replacement. Therefore, this study investigated the effects of replacing dietary FO with a *Schizochytrium* based oil on growth performance, feed efficiency, lipid metabolism and heat stress resistance in Atlantic salmon.

Materials and Methods

A basal diet (S0) containing 25% fish meal and 12% FO was prepared. Five experimental diets were formulated to replace FO with *Schizochytrium* oil at graded levels of 20%, 40%, 60%, 80% and 100% (designated as S20, S40, S60, S80 and S100, respectively). A total of 360 Atlantic salmon (initial mean body weight: 101 ± 0.25 g) were randomly distributed into 18 tanks (450 L) in triplicate groups (20 fish per tank) and fed to apparent satiation three times daily for 6 weeks. Heat stress was induced by adjusting the ratio of cooled water and seawater, resulting in a water temperature range of 16.5–24.9 °C. At the end of the feeding trial, growth performance, feed utilization and survival were evaluated. Blood and tissue samples were collected to determine serum biochemical parameters, non-specific immune responses, antioxidant capacity, digestive enzyme activity, muscle proximate composition, fatty acid composition and hepatic gene expression related to lipid metabolism, stress, pro-inflammation and apoptosis.

Results

Dietary substitution of FO with *Schizochytrium* oil significantly improved growth performance in Atlantic salmon, as indicated by higher final body weight, weight gain and specific growth rate in S20, S40 and S60 groups compared to S0 group. Feed utilization was significantly enhanced, with reduced feed conversion ratio and increased protein efficiency ratio in all FO substituted groups. Survival rate was significantly higher in all FO substituted groups than in S0 group. Serum biochemical parameters were improved, as reflected by significantly lower glucose, triglycerides, aspartate aminotransferase, alanine aminotransferase and low-density lipoprotein levels in all FO substituted groups, while high density lipoprotein level was significantly higher in S60, S80 and S100 groups compared to S0 group. Immune responses were significantly enhanced, with increased myeloperoxidase, lysozyme, nitroblue tetrazolium and total immunoglobulin activities in S40, S60, S80 and S100 groups compared to S0 group. Antioxidant capacity was significantly improved, as indicated by higher superoxide dismutase, glutathione peroxidase and catalase activities, as well as increased total antioxidant capacity in FO substituted groups, while hepatic malondialdehyde level was significantly lower in FO substituted groups. Digestive enzyme activities were significantly increased, with higher trypsin activity in S40, S60, S80 and S100 groups and higher lipase activity in all FO substituted groups compared to S0 group. Muscle DHA and crude protein levels were significantly

(Continued on next page)

higher in substituted groups than in S0 group. Hepatic lipogenesis and lipid transport related genes (*FAS*, *LXR*, *SREBP1* and *SREBP2*) were significantly downregulated in all FO substituted groups. Gene involved in fatty acid desaturation and elongation (*FAD5*, *FAD6*, *ELOV2* and *ELOV5*) showed higher expression, particularly at S100 group. In contrast, lipid catabolism related genes (*AOCX1*, *CPT1*, *PPAR α* , *PPAR β* and *PPAR γ*) were significantly upregulated. Heat shock proteins (*HSP60*, *HSP70* and *HSP90*), pro-inflammatory cytokines (*TNF- α* , *IL-1 β* , *IL-6* and *Mulan*) and apoptosis-related genes (*BAX*, *Caspase3a*, *Caspase7*, *Caspase9* and *P53*) were significantly downregulated, indicating suppressed stress, inflammation and apoptotic responses in FO substituted groups.

Conclusion

In conclusion, replacing FO with *Schizochytrium* oil can improve growth performance, feed efficiency and nutrient utilization by upregulating digestive enzymes and lipid metabolism related genes. Furthermore, dietary substitution enhanced physiological condition, antioxidant capacity and immune responses, while downregulating pro-inflammatory cytokines, stress-related genes and apoptosis-associated genes. These results indicate that *Schizochytrium* oil can effectively improve health status and stress resilience in Atlantic salmon. Based on the weight gain and survival, optimal dietary FO replacement level is estimated to range between 53.5% and 82.4%.

EVALUATION OF PREBIOTIC, PROBIOTICS AND SYNBIOTIC SUPPLEMENTATION IN DIETS FOR BARRED KNIFEJAW *Oplegnathus fasciatus*

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Introduction

Probiotics are live microorganisms that administered in adequate amounts can provide health benefits to the host. In aquaculture, probiotics have been widely used to improve intestinal microbial balance, suppress pathogenic bacteria and stimulate immune responses. Prebiotics are non-digestible feed ingredients that selectively stimulate the growth of beneficial intestinal microorganisms, thereby improving host health. Prebiotics used in aquaculture include fructooligosaccharides, mannanoligosaccharides (MOS), inulin and β -glucans. These compounds are fermented by gut microbiota to produce short-chain fatty acids and other metabolites that support intestinal integrity, nutrient utilization and immune function. Synbiotics (SYN), which are combinations of probiotics and prebiotics have been developed to exert synergistic effects by supplying both beneficial microorganisms and substrates that promote their survival and activity in the gastrointestinal tract. SYN supplementation has frequently shown greater improvements in growth performance, feed efficiency, immunity and stress resistance than probiotics or prebiotics alone, largely through modulation of gut microbiota and enhancement of intestinal barrier function. Barred knifejaw (*Oplegnathus fasciatus*) is a commercially important marine fish species in South Korea, Japan and China. However, its culture is often constrained by stress-induced immune suppression, bacterial and viral diseases. Despite of increasing interest in functional feed additives, information regarding the use of probiotics, prebiotics and SYN in *O. fasciatus* remains limited. Therefore, the present study was conducted to evaluate the effects of dietary probiotics, prebiotic and SYN supplementation on growth performance, feed utilization, digestive enzyme activity, immune response, antioxidant capacity and disease resistance in *O. fasciatus*.

Materials and Methods

A basal diet (Con) containing 45% fish meal (anchovy) and 15% soybean meal was formulated to contain 42% crude protein and 16% crude lipid. Five experimental diets were formulated by replacing starch in Con diet with 0.4% MOS, 0.4% *Lactiplantibacillus plantarum*, 0.4% *Bacillus subtilis*, 0.4% *Bacillus licheniformis* and 0.1% MOS + 0.1% *L. plantarum* + 0.1% *B. subtilis* + 0.1% *B. licheniformis* (designated as MOS, LP, BS, BL and SYN, respectively). A total of 360 fish (initial mean body weight: 116.13 ± 1.01 g) were randomly distributed into 18 tanks (300 L) in three replicates and fed experimental diets for 49 days. At the end of the feeding trial, growth performance, feed utilization, digestive enzyme activity, immune response, antioxidant capacity, serum biochemical parameter, gene expression and disease resistance against *Edwardsiella piscicida* were evaluated.

Results

Dietary supplementation with probiotics and SYN were significantly improved growth performance in *O. fasciatus*, as indicated by higher final body weight, weight gain and specific growth rate compared to Con. Feed utilization was significantly enhanced, with increased protein efficiency ratio and reduced feed conversion ratio, while survival was significantly improved in BL and SYN groups. Digestive enzyme (trypsin and lipase) activities were significantly increased in LP, BS, BL and SYN groups than in Con group. Chymotrypsin activity was significantly increased in MOS, LP and BL groups than in Con group. Lysozyme, myeloperoxidase and immunoglobulin levels were enhanced in probiotics-supplemented groups. Superoxide dismutase activity was significantly higher in MOS, LP, BL and SYN groups compared to Con group. Catalase activity was significantly higher in LP, BS, BL and SYN groups compared to Con and MOS groups. Aspartate aminotransferase and alanine aminotransferase levels were significantly lower in Con, LP, BS, BL and SYN groups than in MOS group. Triglyceride level was significantly increased in SYN group than in Con, MOS, BS and BL groups. Glucose level was significantly increased in BL group than in Con, MOS groups. Gene expression analysis revealed upregulation of pro-inflammatory genes (*TNF- α* , *IL-1 β* , *IL-8*) in LP and SYN groups, while *STAT1* and *STAT3* expressions were downregulated in BS and BL groups than in Con group. Resistance against *E. piscicida* was significantly increased in BS and SYN groups than in Con group.

Conclusion

In conclusion, this study suggested that dietary supplementation with probiotics and SYN improved growth performance, feed utilization efficiency, survival, innate immunity, digestive enzyme activity, antioxidant capacity in *O. fasciatus*. These improvements were accompanied by enhanced immune response and antioxidant capacity, as well as modulation of gene expression related to pro-inflammatory and signaling pathways. Furthermore, probiotics and SYN supplementation increased resistance against *E. piscicida*. These results indicate that probiotics and SYN are effective functional feed additives to enhance growth performance, physiological condition and *E. piscicida* resistance in *O. fasciatus*.

THE IMPACT OF SEA LICE INFESTATION ON THE PROGRESSION OF PISCINE MYOCARDITIS VIRUS INFECTION IN ATLANTIC SALMON (*Salmo salar*), AND VICE VERSA

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Introduction

Piscine myocarditis virus (PMCV) is the causative agent of Cardiomyopathy Syndrome (CMS) (Haugland et al., 2011), a severe viral disease characterized by distinct cardiac inflammation that results in compromised animal welfare and mortality in post-smolt Atlantic salmon (Garseth et al., 2018). At the same time, sea lice (*Lepeophtheirus salmonis*) infestations reduce growth and increase the susceptibility of Atlantic salmon to secondary pathogens due to extensive skin degradation. Mechanical delousing is a common treatment used to physically remove sea lice from fish but often causes increased stress and is associated with CMS outbreaks in PMCV positive populations (Sommerset et al., 2023). Despite these observations the interaction between PMCV infection and sea lice infestation in the same host remains poorly understood. In particular, it is unclear whether sea lice attachment and associated tissue damage influences viral load or the development of cardiac lesions associated with CMS. From an immunological perspective, coinfections may be shaped by trade-offs between antagonistic immune responses. The concept of immunity equilibrium suggests that type 1 responses (to viruses) and type 2 responses (to multicellular parasites) can suppress one another, potentially altering host susceptibility and disease outcomes (Eberl, 2016). Therefore, the objective of this study was to investigate how coinfection with PMCV and sea lice influences viral load, cardiac pathology, and the localized immune response in the heart, as well as whether PMCV infection affects sea lice burden.

Materials and Methods

To assess coinfection, controlled laboratory experiments were performed using post-smolt Atlantic salmon reared in a saltwater recirculation system (RAS). Fish were allocated across four infection groups: a negative control, single PMCV infection, single sea lice (SL) infection, or PMCV + SL co-infection, and sampled biweekly over 14-weeks for tissue sampling or at 3, 8, 22, and 36 days post-infestation (dpi) for sea lice counts. PMCV challenge was administered with an intraperitoneal (IP) injection and sea lice through immersion bath. Total lice counts were conducted to assess sea lice burden during different life-stages on Atlantic salmon in relevant groups. Viral load (PMCV RNA) was quantified via RT-qPCR and cardiac lesion scoring assessed through histopathology. In addition, the immune response in the heart was investigated through gene expression using RT-qPCR targeting anti-viral, cellular-mediated, and anti-inflammatory markers, and cytokine quantification with IL-1 β specific ELISAs to evaluate the early pro-inflammatory response.

Results

Sea lice challenge took place one day before the 7 weeks post challenge (wpc) timepoint – the projected peak of viral load in the heart. Sea lice burden was statistically similar on salmon in the single SL infection and PMCV + SL coinfection group at 3, 8, 22, and 36 days post infestation (dpi). While, viral load in the heart was statistically similar between single PMCV infection and PMCV + SL coinfection groups post-sea lice challenge at 8, 10, 12, and 14 wpc. Expression of genes associated with cellular mediated responses (*MHC-I*, *MHC-II*, *IFN γ* , *Tbet*, *CD8 α* , *GrmA*) were significantly upregulated in the heart of single and co-infected salmon from 6-12 wpc. Although, the expression of antiviral markers (*ISG15 α* and *STAT1*) was significantly higher in coinfecting salmon compared to single infection at 12wpc. In addition, the expression of the anti-inflammatory cytokine *TGF- β* was only significantly upregulated the heart of coinfecting salmon from 8-12 wpc. IL-1 β concentration in the heart was significantly higher in groups experiencing single PMCV and PMCV + SL co-infection at 6 and 8wpc but had reduced to baseline levels in the coinfecting salmon by 10wpc.

Discussion

To date, our results demonstrate that sea lice, which parasitize the skin of Atlantic salmon, have the capacity to modulate the expression of select anti-viral and anti-inflammatory cytokine genes in the heart during PMCV infection. Despite this, when sea lice infect salmon at the peak of viral load in the heart (6-7wpc), there is no effect on viral load at subsequent timepoints up to 14wpc. This suggests that innate antiviral responses triggered early in infection are more important for limiting viral replication at later timepoints, thereby reducing the need for prolonged antiviral gene expression. Increased

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expression of TGF- β in coinfecting salmon indicates a stronger anti-inflammatory response is induced in the heart to resolve cytotoxic and pro-inflammatory activity. This could also help explain the early suppression of IL-1 β in the heart of coinfecting salmon. However, histology results are still pending and will be important for interpreting the transcriptional and cytokine data in relation to tissue-level pathology. To our knowledge, this is the first experimental study describing the effects of sea lice on immune gene expression and IL-1 β concentration in the heart of Atlantic salmon during both single sea lice infestation and virus-parasite coinfection. Collectively, these findings highlight the importance of considering coinfection when evaluating localized immune responses, as well as its implications on fish welfare and the development of improved health management strategies.

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GROWTH PERFORMANCE AND SURVIVAL OF *Holothuria arguinensis* IN INTEGRATED MULTITROPHIC AQUACULTURE WITH *Sparus aurata*: EFFECTS OF FEEDING REGIMES AND PREDATION INTERACTIONS

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Sea cucumbers are highly valued as a traditional delicacy, placing increasing economic pressure on natural populations. Given their ecological role as sediment bioturbators, their incorporation into aquaculture systems offers a promising strategy to alleviate harvesting pressure while enhancing system sustainability. In particular, their function as organic extractors makes them suitable candidates for Integrated Multitrophic Aquaculture (IMTA), where species from different trophic levels are co-cultured to improve resource efficiency. This study evaluated the growth performance and survival of *Holothuria arguinensis* juveniles reared in co-culture with the gilthead seabream, *Sparus aurata*, under different fish feeding regimes that induced different levels of satiety in the seabream, while assessing predation frequency. A total of 90 sea cucumbers (initial mean weight $\pm$ SD: 6.41 ± 7.44 g) were distributed alongside 90 gilthead seabream (175.57 ± 44.66 g) across three feeding treatments: 100% (T1), 75% (T2), and 50% (T3) of the fish biomass, using a commercial feed (EFICO 3053, Biomar). The trial was conducted over 33 days in a 20 m³ flow-through system ($0.5 \text{ L} \cdot \text{s}^{-1}$), partitioned in three sections (one per treatment), all containing sediment and artificial shelters. The specific growth rate ($\% \cdot \text{day}^{-1}$) of *H. arguinensis* and *S. aurata* were determined, and any potential antagonistic predation by *S. aurata* was monitored daily. While *S. aurata* exhibited no mortality and decreasing specific growth rates (SGR) with reduced feeding (0.36, 0.25, and $0.05 \text{ \%} \cdot \text{day}^{-1}$ for T1, T2, and T3, respectively), *H. arguinensis* displayed the highest SGR in T3 ($5.19 \text{ \%} \cdot \text{day}^{-1}$), albeit with the lowest survival rate (16.67%). In contrast, T1 and T2 showed similar SGR values (4.55 and $4.53 \text{ \%} \cdot \text{day}^{-1}$) but higher survival (36.67% and 30%, respectively). The enhanced growth observed in T3 is likely linked to reduced intraspecific competition for organic waste due to lower survival. Notably, predation by *S. aurata* was observed even under full feeding conditions, although individuals exceeding 10 g appeared less vulnerable, suggesting a size-dependent refuge from predation. Overall, the results highlight the potential of *H. arguinensis* as an extractive species in IMTA systems, achieving substantially higher growth rates compared to previous monospecific and co-culture studies. However, the findings also indicate that early-stage juveniles are particularly susceptible to fish predation, emphasizing the need for system designs that optimize waste transfer while minimizing direct interactions during initial growth stages.

Acknowledgment

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COMBINED BIOLOGICAL-PHYSICAL SENSING SYSTEMS FOR OFFSHORE INTEGRATED MULTI-TROPHIC AQUACULTURE

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Introduction

The expansion of aquaculture into more exposed offshore environments is increasing the need for high-resolution environmental monitoring to support safe operations, efficient farm management, and sustainable sector growth (Antonucci & Costa, 2020). This transition is increasingly important as sheltered coastal sites become spatially constrained (Lester et al., 2018). However, environmental monitoring in aquaculture is often based on sparse or intermittent measurements, which can be insufficient to resolve the hydrodynamic and ecological variability that shapes offshore farm performance (Jansen et al., 2016). This study involves an advanced underwater sensing system with acoustic telemetry for remote data offload, diagnostics, and reconfiguration, deployed at a commercial offshore multi-trophic aquaculture site under variable environmental conditions. The deployment and configuration of the system enables the investigation of two open research questions for multi-trophic aquaculture.. First, recent offshore studies have shown the value of acoustically derived observations for examining hydrodynamic–ecological interactions under real-world conditions (Comerford et al., 2025), while field-scale observations of how wild fish use aquaculture sites remain limited (Forbes et al., 2022). Here we explore whether month-long ADCP acoustic backscatter measurements can provide evidence of relative wild fish aggregation and habitat use at an mussel integrated multi-trophic aquaculture (IMTA) farm compared with nearby reference conditions. Second, aquaculture farm structures have been shown to attenuate wave energy in physical modelling studies (Miranda et al., 2025) which could have applications for coastal protection. In this study we aim to investigate these effects through field-scale observations.

Materials and Methods

The study is being carried out at a commercial offshore IMTA site. An array of four acoustically communicating Origin 600 Acoustic Doppler Current Profilers (ADCPs) will be deployed within and around the farm to measure current velocity, wave conditions, turbulence, and acoustic backscatter, enabling comparison of within-farm conditions, structure-associated conditions, and adjacent reference conditions. An idealised layout of the deployment is shown in Figure 1.

The system uses on-board edge computing to distil hundreds of megabytes of raw hydrodynamic data into compact packets containing key environmental metrics, on the order of 100 bytes, enabling efficient near-real-time acoustic transmission to the surface for remote access and system oversight.

The summer 2026 campaign is designed to capture representative operational and environmental conditions at a site exposed to energetic wave forcing and significant tidal range.

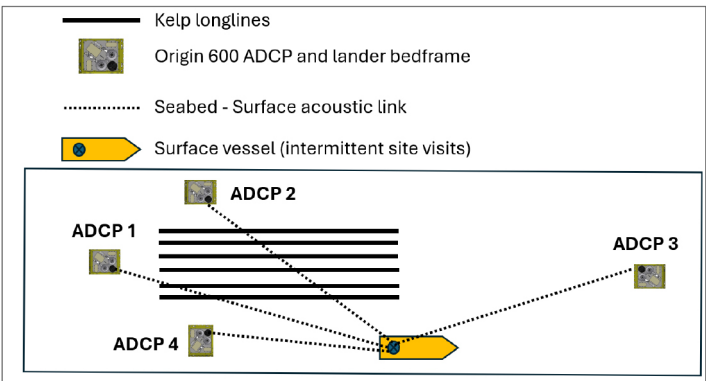


Figure 1 idealised layout of underwater sensor network comprising multiple Origin 600 ADCPs communicating acoustically with a visiting surface vessel, in the presence of IMTA site infrastructure.

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Results and Discussion

As the summer 2026 field campaign will commence after the abstract submission deadline, this abstract will be updated with deployment results in line with conference deadlines. The final results will be used to assess spatial patterns in current and wave conditions across the farm, identify possible flow and wave attenuation effects associated with cultivated biomass and farm structures, and investigate whether ADCP backscatter can provide a useful proxy for wild fish aggregation and habitat use. In doing so, the study will generate preliminary evidence relevant to the hypothesis that offshore aquaculture may provide habitat provisioning benefits, including sheltering functions of potential importance to juvenile and forage fish, with potential relevance to wider questions of habitat value and ocean restoration. The observational framework is expected to strengthen the evidence base for offshore aquaculture monitoring by improving site characterisation, supporting safer operations, and enabling more informed assessment of hydrodynamic and ecological variability at farm scale.

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DEVELOPMENT AND VALIDATION OF A SENSITIVE MOLECULAR ASSAY FOR QUANTIFICATION OF THE SALMON LOUSE *Lepeophtheirus salmonis* IN ZOOPLANKTON SAMPLES

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Introduction

The salmon louse, *Lepeophtheirus salmonis*, is a major ectoparasite of farmed salmon, contributing to substantial production losses through tissue damage, impaired mucosal barrier function, and increased susceptibility to secondary infections. Adverse health outcomes and mortalities are increasing alongside the emergence of treatment resistance and adaptation; therefore, early outbreak detection is essential to facilitate timely control and to support proactive farm management. Conventional monitoring focuses on attached, mature sea lice stages, and the reliability of count estimates is limited by patchy distribution of parasite burden across both hosts and cages, requiring intensive sampling effort. Assessing the density of free-living, planktonic lice stage instead offers a more representative measure of infection risk. However, the larvae’s sparse distribution amongst a diverse background zooplankton community, as well as their morphological similarity to other taxa, makes microscopic analysis a timely process requiring taxonomic expertise. Molecular processing therefore offers a highly specific, high-throughput, and scalable option for the monitoring of free-living sea lice. While species-specific molecular assays exist for *L. salmonis*, comparative studies suggest that they still lack the quantitative accuracy, precision, and sensitivity of visual methods (Bui et al., 2021). Therefore, this study aimed to optimise the molecular workflow by testing novel and existing marker types, amplification methods, and assessing the effects of subsampling depth, DNA extraction efficiency, and background zooplankton density on assay performance..

Methods

We compared existing mitochondrial markers (16S, COI) and novel nuclear high-copy repeat targets, alongside quantitative PCR (qPCR) and loop-mediated isothermal amplification (LAMP) approaches. Spiking experiments were conducted using zooplankton samples containing 0–30 *L. salmonis* larvae (copepodite and nauplius stages), with triplicate replicates per treatment. Samples were homogenised and subsampled (1–3 subsamples per replicate) prior to DNA extraction and target quantification.

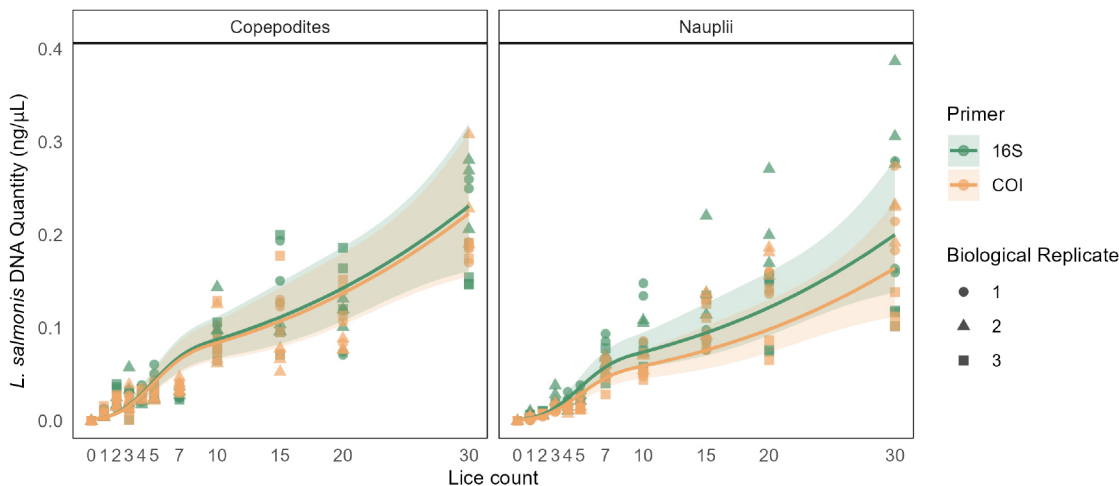


Figure 1. Correlation between qPCR-derived DNA concentration and *L. salmonis* abundance in zooplankton samples. 16S (Krolicka et al., 2022) and COI (McBeath et al., 2006) represent mitochondrial *L. salmonis*-specific molecular markers. DNA signal was significantly lower for nauplii using COI, but not for copepodites ($Z = -5.812, p < 0.001$).

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Results

Target concentration varied non-linearly with lice abundance and DNA extraction yield ($p < 0.001$ for both spline terms). This initial model included a significant life stage $\times$ primer interaction driven by reduced COI signal in nauplii only. A predictive model was then developed to estimate lice abundance from average DNA concentration in the first round of spiked samples. Cross-validated performance indicated reasonable predictive ability with a mean R^2 of 0.82 (SD = 0.11). This corresponded to a mean prediction error of approximately 3.1–3.5 lice (RMSE) across model configurations. Prediction error increased with abundance, with RMSE rising from 1.2 (1–5 lice) to 6.2 (>15 lice). Subsampling depth had no significant effect on model performance, although a minor, non-significant improvement in predictive accuracy was observed with increased subsampling (RMSE: 3.8 $\rightarrow$ 3.2), suggesting diminishing returns beyond a single subsample. Although extraction yield significantly influenced qPCR-derived target concentration, its inclusion did not improve predictive model performance, indicating that normalising samples to account for differential extraction efficiency would not improve estimates.

Implications and ongoing work

While initial findings confirm a strong relationship between qPCR signal and lice abundance, the relative error of 41% at low densities (1–5 lice) could indicate reduced precision in absolute abundance estimates compared to visual methods. However, the method detected signal in all spiked samples (including $n = 1$), whereas traditional microscopy is prone to missing such low densities, suggesting improved sensitivity for early detection. Stage-dependent differences in primer performance highlight the importance of target selection when applying assays to field samples where stage composition is unknown. Development of a novel LAMP-based assay and tandem repeat primer targets for *L. salmonis* are ongoing. A second experimental phase will further validate and compare combinations of marker types and amplification methods under optimal extraction and subsampling conditions using independent samples spiked with realistic and mixed-stage larval densities. Molecular count estimates will be compared against conventional microscopy and automated imaging (PlanktoScope) to assess relative accuracy, sensitivity, and scalability. This work will deliver an integrated, validated molecular workflow for early sea lice quantification in zooplankton, with direct application to improving monitoring sensitivity and supporting proactive management in aquaculture systems.

Acknowledgments

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GROWTH, BIOCHEMICAL COMPOSITION AND LIFE CYCLE ASSESSMENT (LCA) OF TWO SPECIES OF GAMMARIDS (*Gammarus duebeni* AND *Gammarus oceanicus*) AND THE POLYCHAETE *Hediste diversicolor* REARED ON GRASS SEDIMENTS AND FEATHERS

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Introduction

The rapid expansion of salmon aquaculture is increasing demand for sustainable aquafeeds and placing pressure on conventional feed resources (Almås et al., 2023; Miljøstiftelsen Bellona, 2022; OECD/FAO, 2025). Gammarids and polychaetes offer promising circular feed solutions by converting underutilized organic side streams into nutrient-rich biomass (Alberts-Hubatsch et al., 2019; Anglade et al., 2023; Ribes-Navarro et al., 2022; Wang et al., 2019). Yet, knowledge on both their biological performance and environmental impacts remains limited for these novel ingredients to advance from concept to application, due to the absence of industrial-scale production of these species in Scandinavia.

Materials and methods

This study evaluated grass and feather-based diets for gammarids (*Gammarus duebeni* and *Gammarus oceanicus*) and polychaetes (*Hediste diversicolor*), assessing growth, survival, and biochemical composition in small-scale feeding trials. An accompanying life cycle assessment (LCA) compared baseline and alternative feed scenarios for gammarid and polychaete-derived protein meals (1 kg protein), as well as other novel protein sources.

Results and discussion

Both species groups were able to utilize grass- and feather-based diets, but growth rates were limited (1.23 ± 0.39 % d⁻¹ in polychaetes and -0.38 ± 0.10 to 0.50 ± 0.24 % d⁻¹ in gammarids), likely due to limited feed composition, low digestibility and nutrient availability. However, in gammarids, a more digestible grass-sediment mixture performed similarly to the control diet, suggesting that further diet optimization could improve outcomes, while pure grass and feathers demonstrated basic survival potential. Amino acid profiles remained stable in the organisms, and polychaetes retained essential fatty acids (EPA and ARA), indicating potential for trophic upgrading. From an LCA perspective, reduced growth in the alternative feed scenarios compared to baseline scenarios increased rearing times and thus environmental impacts across all categories.

Overall, the results highlight both the potential and current constraints of using circular substrates in low-trophic species production. Further improvements in substrate processing, diet formulation, and system optimization will be necessary to enhance productivity and reduce environmental impacts, supporting the future role of gammarids and polychaetes in sustainable aquaculture feed systems.

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DRYING OF NORWEGIAN RED SEA CUCUMBER (*Parastichopus tremulus*)

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Introduction

Sea cucumbers are considered a delicacy in many Asian countries and are valued for their nutritional and bioactive properties (Bordbar et al., 2011). Dried and processed sea cucumbers can achieve high market prices on the seafood market, depending on species, body size, and product quality (Purcell et al., 2025). Increasing demand has led to overexploitation of wild populations, highlighting the need for sustainable aquaculture. The Norwegian red sea cucumber (*Parastichopus tremulus*) has been identified as a promising cold-water candidate for aquaculture (Landes et al., 2019), with a commercial potential in the Asian market due to its size, meat content, taste, and nutritional content (Kjerstad et al., 2015). To realize this potential, efficient post-harvest processing methods are required to ensure product quality and marketability. Boiling, salting and drying in combination (Purcell, 2014) is the most widely used and commercially important preservation techniques for sea cucumbers, as it stabilizes the biomass, prolongs shelf life, enables storage at ambient temperatures, and reduces transportation costs by producing a lightweight, high-value product suitable for export.

Methods, results and discussion

The reference processing method for sea cucumber, according to Purcell (2014), involves poaching the sea cucumber in seawater at 80°C for 20 min prior to gutting, salting for 2 days, and air drying at 30-40°C. The product is then rehydrated in boiling water for 20 min, followed by a second air-drying step. This procedure was applied to red sea cucumbers (n = 5) to produce a reference product. In addition, vacuum freeze-drying (n = 3) and air drying at 80°C (n = 5) were evaluated as alternative drying methods. The dry matter content in red sea cucumber is low, and between 5-10% (wet basis, wb).

The drying time at 40°C ranged from 24 to 36 h, whereas drying at 80°C required 16 to 24 h. The air velocity over the products was 1.5-1.6 m·s⁻¹, and the relative humidity was 22-27% and 10-15% for drying at 40°C and 80°C, respectively. Vacuum freeze-drying was performed at -51°C and 0.069 mbar for 48 h.

Significant differences in color and rehydration characteristics were observed between the drying methods. The characteristic red color of the Norwegian red sea cucumber was lost during poaching at 80°C. To investigate the effect of processing on color retention, a small-scale experiment was conducted comparing raw sea cucumber (n = 2), salted-only sea cucumber (n = 1), and sea cucumber poached at 40°C and subsequently salted (n = 4). Only the raw sea cucumber retained its characteristic red color.

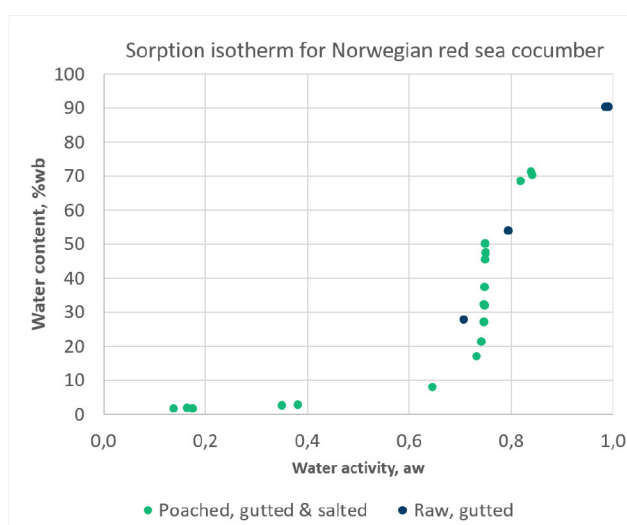


Figure: Norwegian red sea cucumber (*Parastichopus tremulus*) and the sorption isotherm.

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An important factor related to storage and shelf life is product stability. The product-specific sorption isotherm describes the moisture content and storage conditions required to maintain product stability over time. A sorption isotherm illustrates the relationship between moisture content and water activity (aw). Products with an aw below 0.6 - 0.7 are generally stable against microbiological growth, whereas products with an aw between 0.2 and 0.3 are also highly stable with respect to biological, enzymatic, and oxidative reactions.

For Norwegian red sea cucumber, an aw below 0.7 was attained when the moisture content was reduced to less than 10% (wb). To achieve an aw between 0.2 and 0.3, corresponding to maximum product stability, the processed sea cucumber must be dried to a moisture content below 2.5% (wb).

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BREAKING THE PRODUCTIVITY PLATEAU IN *Clarias* AQUACULTURE: CHROMOSOME-SCALE GENOMICS, AI DIAGNOSTICS, AND CLIMATE-SMART SELECTION

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Introduction

The global catfish industry, estimated to be worth over USD 7 billion annually, faces a critical “productivity plateau” driven by unresolved reproductive barriers and climate volatility. While *Clarias gariepinus* × *C. macrocephalus* hybrids dominate production, total male hybrid sterility and declining broodstock quality under thermal stress threaten long-term food security. This work presents a paradigm shift: utilizing the first haplotype-resolved, chromosome-scale genome of *C. macrocephalus* to unlock precision breeding through Genome-Wide Association Studies (GWAS) and AI-based hatchery management.

Materials and Methods

We established an 880 Mb reference genome using PacBio HiFi and Hi-C scaffolding. This genomic blueprint was integrated with: 1) Cytogenetic mapping of meiotic failure in hybrids; 2) GWAS and Genomic Selection (GS) protocols to identify markers for growth and disease resistance; 3) Deep learning (YOLOv11/ResNet18) for automated cranial-based species classification; and 4) Bio-economic modeling of thermal impacts on larval yolk sac absorption efficiency (mg kg⁻¹).

Results

The chromosome-scale assembly (27 pseudo-chromosomes, N50: 33.48 Mb) revealed that satellite DNA divergence (CLA-SAT-149) is the primary driver of meiotic asynapsis, explaining the F₁ hybrid sterility bottleneck. GWAS facilitated the identification of loci associated with the high-growth shooter phenotype, providing a definitive roadmap for genomic selection. Furthermore, a dedicated genome browser with multiple *Clarias* individuals was developed to enable breeders and researchers to perform comparative genomic analysis, execute population-level GWAS, and plan data-driven breeding programs. For industry application, we identified the black testes phenotype as a major cause of hatchery infertility and demonstrated that elevated temperatures accelerate yolk sac depletion, reducing the larval safety window. Our AI framework provided a 99.5% accurate, non-invasive tool for identifying purebred spawners to mitigate stock contamination.

Discussion

Transitioning *C. macrocephalus* from a genomic orphan to a genomic model enables the first implementation of marker-assisted and genomic selection in *Clarias* aquaculture. This integrative framework solves the sterility puzzle, provides AI tools for field-level quality control, and addresses the global fish supply gap through a sustainable, climate-resilient One Health approach.

Acknowledgment

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PRODUCING CARP (*Cyprinus carpio*) IN A THIRSTIER WORLD

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Introduction

Climate change-induced water scarcity is increasingly recognized as a major limiting factor for the sustainability of freshwater pond aquaculture (Boyd and Tucker, 2012). Reduced water availability, elevated summer temperatures, and intensified eutrophication pressure threaten the productivity of traditional carp farming systems across Europe. In pond aquaculture, management interventions that improve oxygen dynamics and ecological buffering capacity are becoming essential to maintain production while minimizing environmental deterioration.

Water depth and aeration are two critical management tools that may mitigate climate-associated production risks. Increased pond depth can stabilize thermal regimes, dilute nutrient concentrations, and improve dissolved oxygen profiles, while supplemental aeration can reduce hypoxia-induced physiological stress (Stone et al., 2014). However, their combined effects on fish growth, water chemistry, stress physiology, and disease dynamics remain insufficiently quantified under commercial semi-intensive carp farming conditions.

This study evaluated the main and interactive effects of pond depth (1 vs. 1.5 m) and aeration regime (nighttime vs. nighttime + daytime aeration) on production performance, water quality, glucose-based stress response, and fish health during the first production season of juvenile common carp.

Materials and Methods

A 2×2 factorial design with two replicates per treatment was conducted over 90 days in eight experimental ponds (470 m² each) at HAKI, Hungary. Treatments included: Water depth: 1 m vs. 1.5 m; Aeration: nighttime only (22:00–10:00) vs. nighttime + 2 h daytime aeration (14:00–16:00). Each pond was stocked with 1260 common carp juveniles and fed standardized commercial diets. Production metrics included total biomass, survival, feed conversion ratio (FCR), specific growth rate (SGR), and final body weight. Environmental parameters included dissolved oxygen (DO), temperature, turbidity, total nitrogen (TN), total phosphorus (TP), chlorophyll a, cyanobacteria, and nutrient ratios. Fish welfare assessments included parasitological and pathological monitoring, while physiological stress was evaluated through plasma glucose and cortisol measurements. Statistical analyses were performed using MANOVA, with significance accepted at $p < 0.05$.

Results and Discussion

Water depth had a highly significant effect on multiple production and environmental parameters. Ponds maintained at 1.5 m depth achieved substantially higher final biomass (mean 252.5 kg vs. 149.1 kg), survival (66.3% vs. 45.1%), and SGR (5.75 vs. 5.15) compared to shallow ponds ($p = 0.01$, $p = 0.01$, and $p = 0.03$, respectively). Deeper ponds also maintained significantly higher dissolved oxygen concentrations ($p < 0.001$), lower turbidity ($p = 0.02$), reduced total nitrogen ($p = 0.04$), and total phosphorus ($p = 0.05$), confirming improved ecological stability.

These findings align with previous observations that greater water volume buffers environmental fluctuations and reduces eutrophication risk in pond systems (Boyd, 2020).

Aeration regime showed less pronounced direct effects on growth performance; however, additional daytime aeration significantly reduced fish plasma glucose concentrations (59.4 mg/dL vs. 67.9 mg/dL; $p = 0.013$), suggesting lower chronic stress exposure. This reduction in glucose indicates improved physiological welfare under enhanced oxygenation, even where biomass gains were not significantly altered. Aeration also significantly influenced TN/TP ratios ($p = 0.04$), reflecting shifts in nutrient cycling and primary productivity.

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Importantly, no statistically significant depth $\times$ aeration interactions were observed for major production indicators such as biomass ($p = 0.23$), survival ($p = 0.12$), or SGR ($p = 0.38$), indicating that pond depth was the dominant production driver. Nevertheless, combined deeper-water and enhanced-aeration systems consistently produced the numerically best outcomes, suggesting additive practical benefits.

Fish health surveillance revealed recurrent parasitic and bacterial challenges, including *Dactylogyrus* spp., *Trichodina* spp., *Aeromonas salmonicida* - associated erythrodermatitis, and severe *Bothriocephalus acheilognathi* infestations with 100% prevalence during peak infection periods. While disease pressure affected all treatment groups, deeper ponds with improved environmental parameters demonstrated better overall resilience and production recovery. The integration of environmental engineering strategies with proactive veterinary interventions appears increasingly essential for climate-resilient pond aquaculture.

Conclusion

This study demonstrates that successful common carp production remains achievable even under reduced water availability and in shallower ponds; however, maintaining greater water depth consistently yields superior production performance, survival, and environmental stability. In scenarios where water scarcity limits the pond's full capacity, maintaining the highest feasible water level is a crucial adaptive strategy to minimize productivity losses. Greater depth offered clear advantages in ecological buffering, water quality, and fish welfare, while supplemental aeration further contributed to reduced physiological stress. Although carp farming can persist under constrained water conditions, these findings highlight that sustaining deeper pond environments whenever possible substantially strengthens resilience, productivity, and long-term sustainability in climate-challenged aquaculture systems.

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SCIENTIFIC AND EDUCATIONAL CENTERS AS DRIVERS OF SUSTAINABLE DEVELOPMENT AND FISHERIES PROMOTION - CENTER FOR FISHERY AND APPLIED HYDROBIOLOGY “MALI DUNAV”

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The continuous growth of the global population, along with increased demand for quality protein sources, has contributed to the rapid expansion of aquaculture, currently one of the fastest growing sectors in food production (Blanchard et al., 2017). In the modern fisheries sector, scientific and educational centres play an important role in knowledge transfer, implementing innovative technologies, and strengthening the link between research and industry.

Their importance is particularly evident in countries such as Serbia, where the consumption of fish and aquaculture products remains substantially below the European average. In this context, the Centre for Fisheries and Applied Hydrobiology “Mali Dunav (Little Danube)” serves as a unique platform integrating scientific research, education, tourism, and the promotion of sustainable use of aquatic resources.

The Centre plays an important role in advancing fisheries and applied hydrobiology by implementing scientific and development projects, providing practical classes for pupils and students, and organising professional meetings, workshops, exhibitions, and training programmes. Particular emphasis is placed on educating children and young people, who, through direct interaction with aquatic ecosystems, are introduced to the principles of biodiversity conservation, the ecological functioning of aquatic communities, and the sustainable management of natural resources.

Beyond its educational and research activities, the Centre for Fisheries and Applied Hydrobiology “Mali Dunav” has significant economic potential through improved production practices, development of professional competences, encouragement of innovation, and promotion of aquaculture as an important economic sector. Furthermore, the Centre contributes to raising environmental awareness and fostering a responsible attitude towards the sustainable use and conservation of natural resources.

Collectively, these factors underscore the need to intensify the promotion of fisheries and aquaculture, increase public awareness of the nutritional and health benefits of fish consumption, and strengthen scientific and educational capacities to support the sector’s sustainable development. Implementing this approach offers multiple benefits, including economic growth, improved public health, the development of sustainable tourism, and more efficient management of natural resources.

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MODELLING HYPOXIA IN E. SEABASS (*Dicentrarchus labrax*): A BIOENERGETIC APPROACH

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Introduction

Oxygen is an important environmental parameter affecting fish growth and health, and is expected to play an increasingly vital role in fish farming in the light of a warming ocean. Furthermore, the transition to a digital future where the industry scales up the adoption of automation and precision, mandates the development of tools that can enhance our predictive capacity regarding the availability of oxygen, or lack thereof, and its effect on the fish, particularly in scenarios of rising temperatures, low water circulation, and local hypoxic events (Wu et al., 2024). In this study we propose a modelling approach based on the Dynamic Energy Budget (DEB) theory, that quantify the potential hypoxic effects on the metabolism of the European seabass (*Dicentrarchus labrax*), explicitly incorporating environmental oxygen as input.

Materials and methods

A previously parametrized and validated DEB model for E. seabass (Stavrakidis-Zachou et al., 2019) was used as the basis for incorporating the effects of hypoxia, with the environmental dissolved oxygen acting as an explicit model input. By adopting the framework of toxicodynamics-toxicokinetics, oxygen deficit was viewed as a stressor via the production of reactive species which act as toxicants and follow certain tolerance thresholds and elimination dynamics. By using literature data on growth and feed consumption under various DO levels, the model was parametrized and a stress factor was applied and evaluated in regards to several modes of actions including effects on feeding, maintenance costs and costs for structural growth. Finally, simulations were performed using in-situ measurements of temperature and DO at the site of the HCMR pilot scale fish farm in Souda bay (Figure 1, left) to showcase model performance along with a scenario of unlimited oxygen supply ($\text{DO} = 7.5 \text{ mg l}^{-1}$) for comparison.

Results

While the development of the oxygen module is ongoing, provisional results offer promising insights. The proposed approach offers realistic outputs as evident by acceptable goodness of fit obtained on the datasets used for parametrization. Evaluation of the different modes of action indicates that effects on feeding, and therefore assimilation, can sufficiently explain hypoxic effects on the metabolism while the inclusion of additional modes of action only marginally improves model fits at the expense of added complexity. Growth simulations based on observed seasonal measurements of DO and temperature at a farm site demonstrate realistic empirical outputs for E. seabass growth (Figure 1). Moreover, comparisons with a hypothetical simulation under unlimited oxygen availability demonstrate observably better growth performance for the latter group suggesting that even small oxygen deficits throughout the production cycle may amount to a cumulative negative impacts on fish growth performance (Figure 1, right).

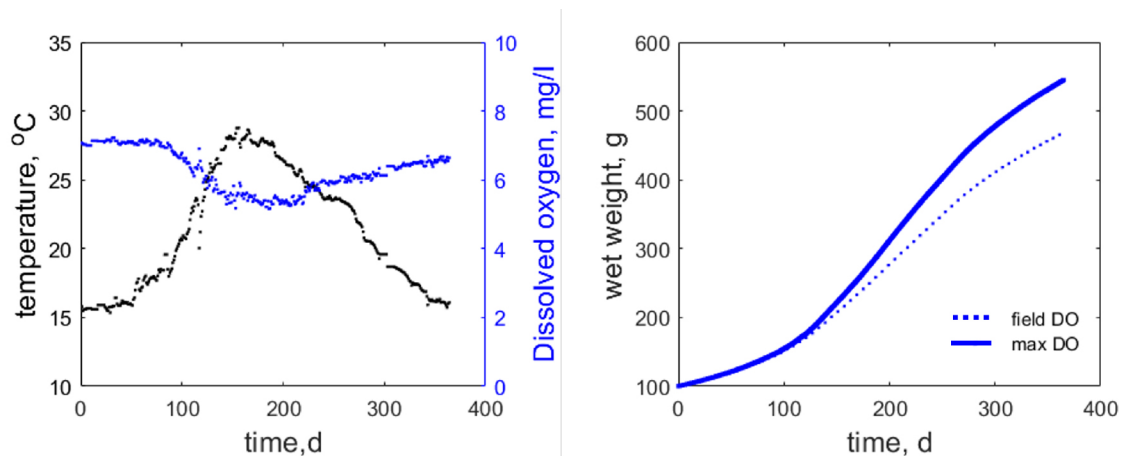


Figure 1. Seasonal changes in temperature and dissolved oxygen (in-situ measurements) at the HCMR farm (left) with the corresponding growth simulations for E. seabass (right) under the actual field DO values (dotted line) and a scenario of unlimited oxygen supply ($\text{DO} = 7.5 \text{ mg l}^{-1}$, solid line)

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DESCRIPTION OF REPRODUCTIVE BEHAVIOUR OF HORMONE-INDUCED AND NON-INDUCED EUROPEAN SEABASS (*Dicentrarchus labrax*) BROODSTOCK FOR ORGANIC AQUACULTURE

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Introduction

The expansion of organic aquaculture demands breeding programs that prioritize natural reproductive competence, as hormonal induction is strictly prohibited under organic certification standards. In the European seabass (*Dicentrarchus labrax*), captive reproduction typically relies on exogenous hormones and *in vitro* fertilisation (IVF) to ensure the number of families required for breeding programs. Therefore, new methods of propagation without the use of exogenous hormones are required to establish and maintain breeding programs for organic aquaculture. However, the behavioural phenotypes associated with spontaneous spawning remain largely uncharacterized. Therefore, this study aimed to characterize and compare the behavioural signatures of both hormone-induced and non-induced seabass broodstock to identify signs of maturation degree. We hypothesized that spontaneous and hormone-induced spawners exhibit behavioural traits that could serve as selection criteria for female ovulation. Here, we present the first assessment designed to indicate the criteria for the development of automated computer vision tools for the identification and selection of ovulated females.

Materials and Methods

To identify breeding signs and traits indicative of female ovulation and male maturity, the spawning behaviour of two groups of seabass broodstock—hormone-induced (HI) and non-induced (NI)—was analysed on the spawning day and the following day. Each group consisted of 10 and 14 males and 5 and 2 females, for HI and NI treatments, respectively. Behavioural observations were conducted during the light photoperiod (approximately 09:30) using Boris software, and the results were subsequently compared between the two groups. Hormonally Induced (HI) fish were maintained under a natural photoperiod and received GnRHa implants at a dose of 20 µg mL⁻¹ for males and 10 µg mL⁻¹ injections for females. In contrast, Non-Induced (NI) individuals were kept under natural light with continuous red light and received no hormonal treatment. The frequency and duration of each behaviour (Table 1) were recorded, alongside any observable morphological signs.

Results

Behavioural differences and similarities were observed between the two groups (Table 2). A single spawning rush was recorded for the NI group, whereas two spawning rushes were recorded for the HI group, all occurring near midday. The predominant behaviour in both tanks was schooling behaviour as the fish continually swam together. Females ready to spawn were distinguishable by pronounced abdominal swelling, which was not observed in any female the day after spawning. In the hours before spawning the predominant behaviour was schooling, which was similar to other schooling periods. However, rub-nudge behaviour started between two to three hours prior to spawning with its frequency increasing as spawning approached. Each spawning rush involved the participation of three to five fish, of which only one was female. The spawning rush was followed by an aggregation up to 2 min minutes after the spawn.

Discussion

This study provides an initial step towards identifying reproductive indicators for organic seabass breeding. Spawning occurred consistently around midday across treatments, lasting less than one minute—a feature that may simplify organic reproduction management. Not all individuals within a tank participated in the spawning events. The most reliable visual cues of gamete maturation were abdominal distension in females and rubnudge behaviour, both evident a few hours before spawning, irrespective of hormonal stimulation. Thus, gravid females with rubnudge behaviour may signal gamete ovulation. Nevertheless, further observations are needed to confirm these patterns and validate them against oocyte sampling in seabass broodstock.

Acknowledgements

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Table 1- Ethogram description of spawning behaviour for marine fish adapted for seabass

Behaviour	Description
Schooling	Fish started to form groups and swim together, following one behind another from one side of the tank to the other
Aggregation	Fish started to form aggregations like a tight ball near the bottom and at one side of the tank
Spawning rush	Female and a male separates from group and swim rapidly in a straight line while close together, male directly behind (at times touching) the female and oriented in the same manner. Rush ends with synchronized gamete liberation, after which the fish separated
Aggregation after spawning	Fish started to form aggregations like a tight ball near the bottom and at one side of the tank almost immediately after spawning
Nose-rudge	Fish approach gravid female and makes physical contact, with mouth, in the lower abdomen near the genital pore

Table 2- Number and duration of behaviour observations of hormone-induced and non-induced Seabass, *D. labrax*, on spawning day and the following day.

Treatment	Non-induced fish				Hormone-induced fish			
Observation day	Spawning day		Day after spawning		Spawning day		Day after spawning	
Behaviour	Number	Duration	Number	Duration	Number	Duration	Number	Duration
Schooling	2	09:30:02	1	10:30:00	3	09:26:36	1	10:25:51
Aggregation	NO	NO	NO	NO	NO	NO	1	00:05:52
Spawning-rush	1	00:00:43	NO	NO	2	00:00:31	NO	NO
Aggregation after spawning	1	00:00:13	NO	NO	2	00:04:02	NO	NO
Rub-nudge	30	NO	NO	NO	40	NO	NO	NO

Number and duration (hours:minutes:seconds) of observations. No observed (NO).

Conclusions

This study used a mechanistic framework to explicitly model the effect of environmental oxygen availability on the metabolism of E. seabass. Preliminary results from model parametrization are characterized by realism while simulations under field and scenario conditions yield interesting insights. Namely, the deviations from the theoretically maximum growth performance simulated under field observations, point to detectable hypoxic effects. Especially under climate change, these highlight the crucial role for close DO monitoring, potential mitigation strategies or adjustment of existing production practices.

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ATLANTIC SALMON *trim25* PARALOGS ANTI-VIRAL FUNCTION IN INFECTIOUS SALMON ANAEMIA VIRUS

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Introduction

Aquaculture is a key food production system supporting the needs of a rapidly growing global population. Despite its promise, the production efficiency and fish health and welfare is negatively impacted by infectious disease. The impact of infectious diseases can be reduced by improving prevention and treatment options, however development of more effective tools requires a deeper understanding of host-pathogen interactions. Using CRISPR-Cas9 genome editing in combination with other sequencing and molecular tools, we interrogated the function of an anti-viral ubiquitin-ligase gene; *trim25*, capable of immune activation. The aim was to deepen our understanding of the regulation of immune response, with the aim of implementing this knowledge for vaccination and selective breeding programs.

Results

Two copies of *trim25* were identified in the Atlantic salmon genome, despite multiple other TRIM25-like annotations. Infection of an Atlantic salmon cell line (SHK-1) with infectious salmon anaemia virus (ISAV) identified unbalanced induction of *trim25* paralog expression, consistent with published results from ISAV infection in addition to POLY (I:C) stimulation, confirming the subfunctionalization of *trim25* paralogs. CRISPR-Cas9 mediated knock-out of paralogous copies of *trim25* confirmed the anti-viral function of Ssa02 *trim25*, but not Ssa12 *trim25*. The antiviral mechanism of *trim25* was confirmed with RNA sequencing of infected knock-out cells. Lastly, over-expression of TRIM25 resulted in the reduction of viral replicaton in SHK-1 cells, confirming the anti-viral role of *trim25*.

Discussion

Taken together, these results confirm that *trim25* is a anti-viral E3 ubiquitin ligase, which conserves the mechanism characterized in model species; namely regulation of the RIG-I like receptor pathway. *Trim25* has previously been identified as a putative gene behind a QTL for resistance to ISAV in Atlantic salmon, and as such further understanding of this gene presents an opportunity to select for more resistant fish. Additionally, the toolset developed in this project offers the opportunity to interrogate gene function in Atlantic salmon.

QUALITY IN SALMON EXPORTS: EVIDENCE FROM CHILEAN AQACULTURE

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Exports of fresh high value agricultural products, such as fresh fish and fruits, represent an increasing share of global food trade. The highly perishable nature of such products makes them less suitable for bulky transport to reduce transportation costs than more traditional agricultural commodities like grains or preserved foods. One will expect exporters to adjust their trading activities accordingly to ensure the quality and accessibility of the products in the final markets, as this commands the highest prices. This paper investigates the role of product quality in exports of fresh farmed salmon from Chile, a highly perishable product for which exports has increased strongly since the turn of the century. Utilizing customs data for the period 2012-2023, we first demonstrate that estimated structural quality closely aligns with physical quality indicators in exports of fresh salmon. Secondly we test the Alchian-Allen hypothesis by comparing quality distributions across transport modes. We find that air freight is systematically associated with higher product quality values compared to low-cost alternatives. Finally we disentangle exports values into respective trade margins documenting that quality primarily operates through scale (the intensive margin) in Chilean salmon trade. Our results provide empirical evidence of how product quality affects global agri-food supply chains.

REMOVE, RETAIN, OR REPURPOSE? ENVIRONMENTAL PERSPECTIVES ON DECOMMISSIONING OFFSHORE STRUCTURES

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Projects must take the ending into account from the very beginning. The decommissioning phase for offshore structures has begun and many questions arise. Requirements and effort will multiply sharply. The rapid expansion of offshore industries, particularly offshore wind energy, has led to an increasing number of installations that will require decommissioning over the coming decades. This is a learning tool for any offshore activity, such as offshore aquaculture. Selecting an appropriate decommissioning strategy is a complex process that must balance environmental, technical, economic, and regulatory considerations. While complete removal has traditionally been regarded as the preferred option, alternative approaches such as partial removal or leaving structures in situ are receiving increasing attention due to their potential ecological and economic benefits. This poster reviews the current state of knowledge on the environmental impacts of different decommissioning scenarios for offshore wind turbines while highlighting the repurposing potential for offshore aquaculture. Particular attention is given to impacts on hard substrate habitats, artificial reef communities, biodiversity, sediment dynamics, water quality, carbon emissions, and material recycling. The review also points out current knowledge gaps and discusses how environmental evidence can support sustainable, site-specific decommissioning decisions.

Introduction

The shared use of the same marine space by offshore wind and aquaculture is becoming an increasingly important solution and is progressing rapidly. Many offshore structures approach the end of their operational lifespan, decommissioning has emerged as an important environmental and management challenge. Traditionally, regulations have favored complete removal of offshore infrastructure to restore the seabed and minimize long-term liabilities. However, offshore structures provide multiple repurposing options and often function as artificial reefs, supporting diverse biological communities and providing ecosystem services. Consequently, alternative decommissioning options, including partial removal or leaving selected components in place, are being considered. Understanding the environmental consequences of these different scenarios is essential for developing sustainable decommissioning strategies.

Materials and Methods

This study is based on a comprehensive review of existing decommissioning case studies, planned decommission scenarios for upcoming cases, peer-reviewed scientific publications, regulations, industry reports, and stakeholder interviews. Literature addressing offshore wind energy and offshore aquaculture was evaluated to compare the environmental outcomes of complete removal, partial removal, and in situ decommissioning. The review focused on ecological impacts, including changes in benthic communities, fish assemblages, habitat complexity, sediment disturbance, contaminant mobilization, changes of local hydrodynamics greenhouse gas emissions associated with decommissioning operations. Similarities and differences between offshore wind and aquaculture infrastructure were also examined.

Results

The literature demonstrates that each decommissioning scenario presents distinct environmental advantages and disadvantages. Complete removal generally maximizes material recovery and facilitates seabed restoration but often causes significant short-term disturbance through sediment movement, underwater noise, and disruption of established benthic habitats. Partial removal can substantially reduce these impacts while preserving artificial reef communities that have developed over years or decades. They also can provide mooring points for offshore aquaculture and monitoring measures. However, retaining infrastructure may introduce long-term challenges, including structural degradation, potential contaminant release, navigational considerations, and continued monitoring requirements. The environmental performance of each option depends strongly on local ecological conditions, water depth, substrate type, species composition, and the characteristics of the offshore installation. Overall, the reviewed studies indicate that environmental outcomes are highly site-specific and cannot be generalized across regions or infrastructure types.

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Discussion

The findings emphasize that decommissioning decisions should not rely on a single default approach but instead be guided by comprehensive, site-specific environmental assessments. Incorporating ecosystem functioning, biodiversity conservation, life-cycle environmental impacts, and circular economy principles into decommissioning planning can improve long-term sustainability. Advances in ecological monitoring, habitat mapping, and decision-support tools will further strengthen evidence-based management. Future research should focus on long-term ecological responses following different decommissioning strategies, particularly for offshore wind farms and aquaculture facilities, to reduce existing uncertainties and support adaptive regulatory frameworks. Developing standardized environmental assessment methodologies will be essential for balancing ecosystem protection, resource efficiency, and socio-economic objectives in the sustainable management of offshore infrastructure at the end of its operational life.

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FLOW IMAGING MICROSCOPY IN AQUACULTURE: HARMFUL ALGAE MONITORING AND SHELLFISH HATCHERY APPLICATIONS

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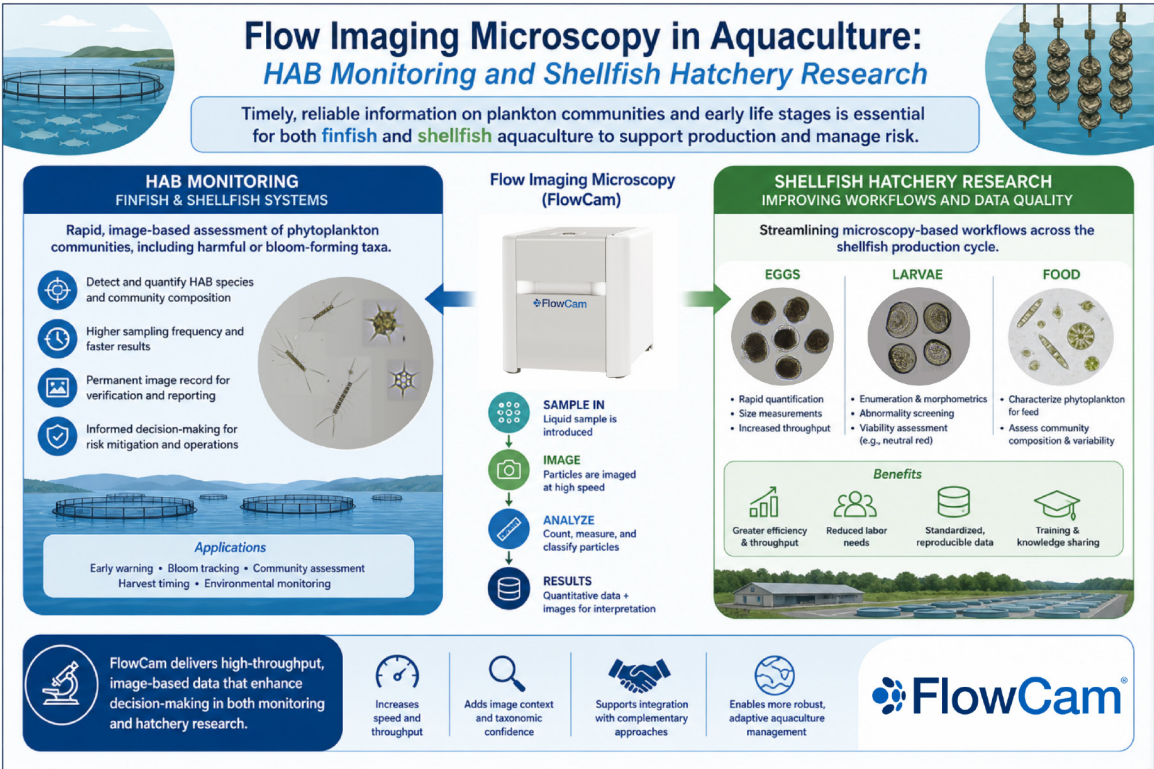
Aquaculture systems, spanning both finfish and shellfish production, depend on timely, reliable information on plankton communities and early life stages to support both operational decision-making and risk management. Monitoring harmful algal blooms (HABs) and assessing hatchery performance remain methodologically challenging, as traditional microscopy is time-intensive and often limits throughput, while single-parameter approaches do not fully capture biological variability. As a result, complementary analytical techniques are increasingly used to improve both environmental monitoring and hatchery workflows.

Flow imaging microscopes (FIM) like FlowCam enable the rapid imaging, enumeration, and measurement of particles in liquid samples while preserving a visual record of each object. In aquaculture contexts, this approach supports **HAB monitoring across finfish and shellfish systems** by providing quantitative and image-based insights into phytoplankton community composition, including the detection of bloom-forming or harmful taxa. These datasets enable more consistent monitoring and support faster decision-making compared to conventional methods.

At the same time, FIM is being adopted in **shellfish hatchery research** to streamline microscopy-based workflows and expand the scope of data collection. Applications include egg quantification, larval enumeration and morphometrics, and the characterization of phytoplankton used as feed. Imaging-based analysis increases throughput while generating standardized datasets that support reproducibility, training, and retrospective analysis. Ongoing method development efforts, including approaches to assess larval viability, aim to further extend the utility of the technique in hatchery settings.

This presentation introduces the role of FlowCam in aquaculture, outlines practical considerations for its implementation across monitoring and hatchery applications, and presents case examples illustrating its use in HAB assessment and shellfish production workflows. By positioning FIM within a broader analytical framework, we highlight its potential to enhance data richness, improve efficiency, and support integrated monitoring strategies across aquaculture systems.

Table/Figure 1



A COMPARATIVE ANALYSIS OF GENETIC RESISTANCE TO WHITE SPOT SYNDROME VIRUS, HEPATOPANCREATIC NECROSIS DISEASE AND INFECTIOUS HYPODERMAL AND HEMATOPOIETIC NECROSIS VIRUS AND ITS RELATIONSHIP WITH GROWTH TRAITS IN *Penaeus vannamei* UNDER COMMERCIAL FARMING CONDITIONS

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Introduction

The farming of *Penaeus vannamei* is one of the cornerstones of the global aquaculture, particularly in countries such as Ecuador, where it is one of the main exports. However, diseases remain one of the factors limiting the sector's sustainability and profitability. Among these, the White Spot Syndrome Virus (WSSV) stands out for its high virulence and mortality rate, whilst acute hepatopancreatic necrosis disease (AHPND), caused by *Vibrio parahaemolyticus*, results in significant economic losses in intensive systems (Negm, 2024; Martínez Soler et al., 2025). Furthermore, the Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV), although less lethal, affects growth and the commercial quality of the product (Shin et al., 2025). In this context, genetic improvement programmes have proven to be an effective tool for increasing disease resistance. However, the relationship between resistance and production traits may vary depending on the pathogen.

Given the impact of infectious diseases on shrimp aquaculture, the aim of this study is to compare the genetic parameters for resistance to WSSV, AHPND and IHHNV, as well as their correlations with growth traits, using data from the PMG-BIOGEMAR® programme, with a view to providing information that can be applied to the design of selection strategies for these traits under industrial conditions.

Materials and Methods

Data from various trials conducted on different generations of *Penaeus vannamei* within the PMG-BIOGEMAR® breeding program in Ecuador were used. The growth-oriented selection scheme based on weight is founded on BLUP methodology and the optimal contribution selection (OCS) (Meuwissen, 1997). For WSSV, approximately 4,700 individuals weighing approximately 2 g, from 220 families of generation 7 (G7), were analysed. These were subjected to an experimental challenge via oral infection with two viral strains (pBIO and pCENA) under different salinity conditions (5 ppt and 35 ppt), with survival and viral loads assessed using qPCR (Negm, 2024). In the case of AHPND, two challenges were carried out via immersion (Test 1) and oral (Test 2) infection with *Vibrio parahaemolyticus*. Approximately 3,350 shrimps weighing approximately 0.8 g and 3.5 g, from 159 families of generation 5 (G5), were used; growth parameters, survival rates and infection levels were recorded (Martínez Soler et al., 2025). For IHHNV, data on natural infection were analysed for around 11,500 individuals at harvest size (approximately 25 g) under industrial farming conditions. These animals were originated from 120 families of generation 4 (G4), and growth, survival and viral load were assessed using qPCR (Shin et al., 2025). The variance components and genetic parameters (heritabilities and genetic correlations) were estimated using mixed models based on BLUP and restricted maximum likelihood (Neumaier and Groeneveld, 1998; Groeneveld et al., 2010; Miształ, 2022).

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Results and discussion

The results showed differences in the genetic architecture of resistance to the three diseases studied. Resistance to WSSV exhibited low heritability and a negative genetic correlation with growth traits (-0.43 REML, -0.44 BAYESIAN), indicating a trade-off between resistance and production (Negm, 2024). In contrast, resistance to AHPND showed low to moderate heritabilities and positive genetic correlations with growth traits, reaching values above 0.55 under experimental conditions (Martínez Soler et al., 2025), suggesting that indirect selection for weight could improve resistance. IHNV showed low to moderate heritabilities, and its genetic correlations with growth traits were virtually zero with the viral load (0.01–0.05), and low with survival (0.22–0.29) (Shin et al., 2025), indicating a limited impact on production performance.

Overall, the results show that the relationship between disease resistance traits and growth is pathogen-dependent; therefore, there is no single selection strategy. These findings have direct implications for the industry, as they enable the design of specific selection strategies based on the predominant health risk in each production system. In this context, the need to develop multi-trait selection indexes that integrate disease resistance and growth traits is reinforced, thereby enabling the simultaneous optimization of robustness and productivity.

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SHARED MOLECULAR TARGETS OF MAJOR SPIRULINA-DERIVED BIOACTIVE COMPOUNDS IN *Danio rerio*: INSIGHTS FROM PROTEOME-WIDE INVERSE DOCKING

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Introduction

Spirulina (*Arthrospira platensis*) supplementation has been associated with a range of beneficial effects in fish, including modulation of immune responses, antioxidant defence, metabolism, growth and reproduction (Oviedo-Olvera et al., 2025). However, the molecular mechanisms underlying these effects remain poorly understood. Here, we investigated the potential molecular targets of three major spirulina-derived bioactive compounds, phycocyanobilin (PCB), β -carotene and γ -linolenic acid (GLA), using a proteome-wide inverse docking approach in *Danio rerio*.

Materials and Methods

Inverse docking was performed against the *D. rerio* proteome using the ProBiS-Dock platform (Konc et al., 2022). Predicted targets were consolidated at the protein level and evaluated based on their docking scores and recurrence across the three compounds. Shared targets were further characterised, and STRING MCL clustering was performed using the combined set of proteins identified as potential targets of the three spirulina-derived compounds. A literature search was also conducted to identify experimental evidence for interactions between the three compounds and the predicted target proteins.

Results

The three compounds differed in the number of predicted protein targets. Across all three compounds, 73 unique protein targets were identified. GLA showed the broadest predicted target profile, with 73 targets, compared with 17 for PCB and 15 for β -carotene. Despite these differences, 10 proteins were shared by all three compounds. Seven of these showed relatively favourable docking scores across the three compounds, with six consistently ranking among the top 10 predicted targets. The shared targets were associated with processes including cell-cycle regulation, apoptosis, cellular stress, metabolism and immune-related functions. Protein-protein interaction analysis of the combined target set revealed several functional clusters, with the largest involving proteins associated with autophagy, while additional clusters were related to pore-complex function, steroid metabolism, purine/urate metabolism and TRP channels. The literature-based assessment revealed only limited experimental evidence for direct interactions between the selected compounds and proteins, and the available evidence came exclusively from non-fish experimental systems.

Discussion

The recurrence of several protein targets across all three compounds suggests that chemically distinct spirulina-derived compounds may partly converge on shared cellular processes rather than acting through entirely independent mechanisms. The overlap with genes reported to respond to dietary spirulina supplementation provides additional biological context for these predictions, although it does not demonstrate direct regulatory relationships. The limited experimental evidence, particularly the lack of studies in fish, highlights the need for targeted validation of the predicted compound–protein interactions. Overall, this study provides a proteome-wide view of potential molecular targets of major spirulina-derived compounds and generates testable hypotheses about how individual compounds may contribute to the broader biological effects of spirulina in fish.

Acknowledgement

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PRELIMINARY RESULTS OF THE ANALYSIS OF THE HUNGARIAN FISH VALUE CHAIN AND ITS POSITION IN EUROPEAN UNION CONTEXT

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The fisheries Data Collection Framework (DCF) project at the Institute of Agricultural Economics (AKI) supports the monitoring and analysis of the Hungarian fish value chain. Data are collected from key actors along the value chain to better understand its structure, dynamics and economic performance. The project covers the collection and analysis of data from fish producers (farm-gate fish sales by sales channel), fish prices in consumer markets and retail chains, prices of fishmonger websites, the activities of fish processors, and foreign trade in fish and fish products. Together, these data provide an overview of market flows, pricing and the main links between producers, processors, traders and consumers.

In our analyses, we sought to explore the key characteristics and interrelationships of the Hungarian fish value chain, including the links between its different stages, and to examine its position within the broader EU context.

The information generated through this process helps stakeholders gain a more accurate understanding of market dynamics, supply and demand, as well as both short- and long-term production and marketing trends. These data not only support informed business decision-making but are also indispensable for the development of agricultural policies and support measures.

The Hungarian fish consumption is relatively low (6.1 kg per capita in 2024) by international standards, remaining well below both the EU (22.89 kg per capita in 2023) and global averages (21.1 kg per capita in 2023, FAO, 2026). However, a closer analysis of the composition of fish consumption reveals important and interesting patterns in consumer preferences and the structure of domestic demand.

However, it is noteworthy that in terms of freshwater fish production per capita, Hungary ranks first among EU Member States. As a landlocked country, Hungary's freshwater aquaculture sector is particularly important, providing significant ecosystem services and contributing to water retention and management, especially in increasingly drought-prone years. Ensuring appropriate support for this strategically important sector is therefore essential.

Pond farms produce approximately 14,000 tonnes of fish per year intended for human consumption, mostly common carp (*Cyprinus carpio*), which accounts for around 11,500 tonnes, representing around 80% of the production. The volume of fish produced in intensive systems reached 4,400-4,500 tonnes of which more than 90% was African catfish hybrid (*Clarias gariepinus* x *Heterobranchus longifilis*). Based on our preliminary findings, pond farm's main sales channels for common carp are wholesalers, processors and retailers, followed by exports and the recreational fisheries market, while African catfish hybrid is processed and sold mainly by the producers themselves. Despite the relatively high level of freshwater fish production per capita, around 70% of fish consumption is supplied by imports from the EU, of which approximately 70% consists of preserved and canned fish products. At the same time, only around 30-40% of domestically produced fish is processed by Hungarian fish processors, of which approximately 70% is African catfish hybrid and only around 20% is common carp.

An important milestone in the coming period will be the assessment of aquaculture production associated with recreational fisheries, including the launch of data collection on the production activities operated by recreational fishing organisations, making this important segment of the Hungarian fisheries sector more visible and suitable for comprehensive analysis.

We would also like to draw attention to the Agricultural Information Portal (AIP), maintained by AKI and freely accessible to all users. The portal provides production-related data and market information for stakeholders and the general public. By making reliable information readily available, the portal contributes to increasing the transparency of the Hungarian fish value chain and supports evidence-based decision-making.

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Acknowledgment

The DCF project (MAHOP_PLUSZ-1.2.1-24-2024-00001) is implemented within the framework of the Hungarian Fisheries Operational Programme Plus, with the support of the European Union and co-financed by the Hungarian State. This project, to be implemented during the 2021–2027 EU development period, is one of the operations of strategic importance designated by Hungary, known as ‘Pillars’. The Pillars play a key role in achieving development policy objectives, ensuring the successful implementation of the relevant operational programme, and the effective and transparent use of EU funds.

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DIGITIZING THE AQUACULTURE VALUE CHAIN: A MODULAR PLATFORM MODEL FOR MARKET EFFICIENCY, INTELLIGENCE AND RISK MANAGEMENT IN INDIA

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Introduction

India is the third largest aquaculture producer globally, contributing approximately 8% of world production and generating over USD 7.6 billion in seafood exports annually (FAO, 2022; MPEDA, 2023). The sector supports over 28 million livelihoods and is central to India's Blue Economy policy agenda under the Pradhan Mantri Matsya Sampada Yojana (PMMSY), which has committed INR 20,050 crore to fisheries development through 2025 (Government of India, 2020). Despite this scale and policy priority, the sector's value chain remains structurally fragmented: input procurement, market intelligence, buyer-seller linkages, and risk management operate in silos, with the majority of transactions conducted informally and offline (World Bank, 2022). This fragmentation suppresses farmer income, limits supplier reach, increases information asymmetry, and elevates operational risk. While digital platform models have significantly improved market efficiency in comparable agricultural sectors, including crop inputs (DeHaat, AgriBazaar) and dairy (StellApps, Stellapps Technologies), aquaculture-specific B2B digital infrastructure remains largely underdeveloped globally (Trendov et al., 2019). The collapse of eFishery in Indonesia in 2025, previously valued at USD 1.4 billion, further underscores the need for financially disciplined, modular approaches to aquaculture digitization. This paper presents Aquaex as a case study in platform-based value chain digitization for emerging aquaculture markets.

Platform Architecture and Methodology

Aquaex was designed as a modular B2B operating system integrating four functional layers to address distinct but interdependent inefficiencies across the aquaculture value chain. First, AquaNews provides an industry intelligence module aggregating market price signals, regulatory updates, export trends, and sector developments, functioning as a trust-building and decision-support tool for supply chain participants (Shepherd & Achterberg, 2020). Second, Aqualyst is a verified B2B marketplace enabling structured procurement of aquaculture inputs (feed, seed, chemicals, equipment) and post-harvest buyer linkage, incorporating single, bulk, and group ordering mechanics with cash-on-delivery as a deliberate trust mechanism. Third, a mandatory multilingual communication layer (Wisemelon) enables native-language coordination via WhatsApp-based interfaces, addressing communication barriers across India's linguistically diverse aquaculture regions, a design principle aligned with evidence on mobile-first adoption in low-connectivity agricultural markets (GSMA, 2023). Fourth, Aqua Insurance, delivered in partnership with Trovity, provides aquaculture-specific risk coverage including crop loss, disease, and environmental risk directly accessible through the platform, responding to documented underinsurance in the sector (Swiss Re Institute, 2021). Platform development followed a capital-light methodology using modular no-code infrastructure, prioritising rapid deployment, iterative improvement, and low adoption barriers across a heterogeneous farmer and supplier base.

Results and Early Outcomes

The Aquaex platform was launched with all four modules live and demonstrated at Aquaex Lucknow 2026 (February 27–28, 2026), a sector expo attracting participation from aquafarmers, input suppliers, processors, and exporters across nine Indian states. The platform currently serves verified suppliers and B2B subscribers across the aquaculture input and output value chain. Early adoption indicates strong engagement from mid-to-large aquaculture businesses seeking structured procurement and verified market intelligence. The mandatory Wisemelon onboarding model has demonstrated measurable impact on communication completeness and native-language accessibility. The integrated insurance offering has begun generating uptake through the Trovity partnership. The platform's modular architecture has enabled rapid iteration without significant capital expenditure, validating the capital-light deployment model for emerging aquaculture markets and demonstrating an alternative growth trajectory to the hardware-heavy and VC-dependent models associated with platforms such as eFishery (Clarkson & Garlock, 2022).

(Continued on next page)

Discussion

The Aquaex model demonstrates that modular digital infrastructure combining intelligence, commerce, communication, and insurance can address the multi-layered inefficiencies characteristic of high-production, low-digitization aquaculture markets. The platform's transaction data architecture is designed to generate longitudinal insights with direct implications for improved insurance underwriting, input financing, and evidence-based policy design, outcomes aligned with the digital transformation agenda articulated in FAO's Blue Transformation roadmap (FAO, 2022b). Key operational challenges identified include supplier verification at scale, cold-chain logistics integration, and digital literacy constraints in low-connectivity regions. The paper discusses design responses to each of these constraints and their transferability to comparable markets in Southeast Asia and the Gulf region. The presentation concludes with an expansion roadmap covering planned entry into the United Arab Emirates and Southeast Asia, and discusses conditions under which this modular platform model could be replicated across other high-production, low-digitization aquaculture economies.

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FROM LAB TO FARM – BLOOD BIOCHEMISTRY AS A PRACTICAL TOOL IN FISH HEALTH MANAGEMENT

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Fish health is central to farming, yet assessments are mostly indirect through detection of pathogen presence, environmental conditions, and performance indicators such as growth, feed intake, and mortality. Such data tells the farmer what has already happened and lack direct input from the farmed animals.

Blood biochemistry changes that. It is a key prognostic tool in human medicine and a cornerstone of veterinary health management. It is routinely used to provide direct insight into organ function, metabolic status, disease, and treatment response. The same approach can be applied for routine fish health monitoring because it provides direct physiological insight into how fish respond to their environment and challenges associated with it.

Over the past decade, WellFish Tech has developed, piloted, and commercially validated, blood biochemistry and modelling as routine tools for on-farm fish health assessment tools. Case studies and commercial examples have demonstrated the value of the tools in three areas: continuous health monitoring, mortality forecasting and health modelling. The outputs of the forecast and models support informed decision-making, helping optimize farm management, reduce losses, and improve financial performance. More importantly, these outputs shift the health management framework from reactive to proactive and provide a glimpse into the future state of the animals and opportunity to intervene before losses occur.

This presentation will demonstrate how blood biochemistry works at a farm level and what health modelling and mortality forecasting mean for farm management and animal welfare.

VALIDATION OF MOLECULAR SEX MARKERS ACROSS THREE STURGEON SPECIES REVEALS SPECIES-DEPENDENT DISCORDANCES

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Introduction

Sturgeons (Acipenseridae) are among the most endangered vertebrate groups and represent one of the most valuable aquaculture species due to their production of high-value caviar and meat (Bronzi et al., 2019). These long-lived fish exhibit late sexual maturation, with females often reaching reproductive maturity several years later than males, while lacking external sexual dimorphism until advanced gonadal development (Bronzi & Harald Rosenthal, 2014; Fajkowska et al., 2020). Consequently, early sex identification is of major economic and management importance, allowing producers to optimize broodstock management, reduce the costs associated with rearing non-productive males for caviar production, and improve breeding and conservation programmes. Current sexing methods, including ultrasonography and endoscopy, are generally applicable only in subadult or adult fish (Fajkowska et al., 2020). Recent genomic studies identified a conserved female-specific genomic region supporting a ZZ/ZW sex determination system in sturgeons and led to the development of PCR-based molecular markers, including AllWSex2 (AllSex), reported to be conserved across multiple sturgeon species (Kuhl et al., 2021). Subsequent studies proposed additional sex-associated markers, such as SSM, to improve molecular sex identification, highlighting the potential of DNA-based assays for non-invasive early sexing in aquaculture (Ruan et al., 2021) . Therefore, independent validation of these molecular assays across different species and breeding populations remains essential before their routine application in aquaculture.

Material and methods

Fin clips were collected from Siberian sturgeon (*Acipenser baerii*, n. 119), Russian sturgeon (*Acipenser gueldenstaedtii*, n.62) and white sturgeon (*Acipenser transmontanus*, n.62). Sex was determined by ecography as routine procedure. Genomic DNA was extracted using a modified CTAB protocol. Molecular sexing was performed by PCR using two female-specific markers previously described in the literature: AllWSex (AllSex) (Kuhl et al., 2021) and SSM4 (Ruan et al., 2021). The AllWSex assay was performed in multiplex with the mitochondrial cytochrome b internal amplification control using GoTaq® Hot Start Colorless Master Mix (Promega) under the optimized conditions described by Kuhl et al. (2021), with minor modifications to primer concentrations. In case of disagreement between the ecography and the AllWSex assay , the SSM4 marker was amplified following the protocol reported by Ruan et al. (2021). PCR products were resolved by electrophoresis on 1.2% agarose gels and visualized under UV illumination.

Results

The AllWSex marker showed complete agreement with ultrasonographic sex determination in all *Acipenser baerii* and *A. gueldenstaedtii* specimens. In *A. transmontanus*, molecular and ultrasonographic sex assignments were concordant for the majority of samples; however, 9% of the samples classified as males by ecography yielded the female-specific AllWSex amplification pattern. These discordant samples were subsequently analysed using the SSM4 marker, which also identified all three specimens as females. No discrepancies were observed among female individuals (Table 1).

Species	Females (+)	Males (-)	Discordant	Total
<i>A. baerii</i>	58	61	0	119
<i>A. gueldenstaedtii</i>	31	31	0	62
<i>A. transmontanus</i>	31	28	3	62

Table 1. Results of the AllWSex assay in the three sturgeon species. Females were positive for the female-specific marker, whereas males were negative. Discordant samples were ecographically identified males testing positive for the female-specific marker.

(Continued on next page)

Discussion

Although AllWSex agreed with ultrasonographic sexing in *Acipenser baerii* and *A. gueldenstaedtii*, discrepancies in *A. transmontanus*, confirmed by the independent SSM4 assay, indicate that current molecular markers may not be universally applicable across sturgeon species. These species-dependent limitations highlight the need for species-specific assays to improve early sex identification and reduce false-positive results in aquaculture that could lead to significant economic losses for commercial farms.

Acknowledgment

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ASSESSING THE INFLUENCE OF FEED METHOD ON BALLAN WRASSE (*Labrus bergylta*) WELFARE AND DELOUSING EFFICIENCY

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Introduction

Ballan wrasse (*Labrus bergylta*) is a significant cleaner fish species used as a biological control in Atlantic salmon (*Salmo salar*) aquaculture against ectoparasitic sea lice (*Lepeophtheirus salmonis*) (Overton *et al.*, 2019; Torrissen *et al.*, 2013). The relationship between rearing conditions, ballan wrasse-specific welfare, and delousing efficiency are poorly understood, thus presenting the opportunity to identify optimal husbandry practices to promote the development of effective cleaner fish.

Materials and Methods

This study included the allocation of three treatments to 600 fish across nine replicate tanks. A pellet-only diet was used as the control, with treatments consisting of feeding blocks only or a combination of blocks and pellets. The rationale behind using feeding blocks is to imitate and promote the “nipping” action required for their role in sea lice management. The wrasse were exposed to their treatments across a 16-week period with data collection occurring at the baseline and three-week intervals. At every interval, neophobia tests were conducted with a LEGO novel object to analyse boldness as a behavioural trait associated with delousing. Growth and welfare were assessed via morphometric measurements and adapting pre-existing operational welfare indicators (OWIs) to be applied in a species-specific context (Bosakowski & Wagner, 1994; Hoyle *et al.*, 2007; Geitung *et al.*, 2020; Treasurer & Feledi, 2014). Gut microbiome samples were taken to provide insights into the effect of feed method on physiological condition. Welfare and morphometrics were sampled at the baseline, week 10, and week 16, with microbiome data representing the baseline and week 16.

Analysis

Data analysis is currently ongoing via mixed effects modelling, including feeding treatment as a fixed effect whilst accounting for tank-level variation. Analysis incorporates the differences between treatments as well as across the 16-week exposure period. Behavioural factors assessed include number of contacts, proximity to, and latency to make contact with the novel object. Morphometrics, Fulton’s condition factor, and OWI scores provide the insights into implications to growth and physical welfare. 16S sequencing will examine differences in gut microbiome.

Discussion

By integrating behavioural, morphometric, welfare, and microbiome measures, this study identifies and enhances ballan wrasse-specific OWIs, addressing a current gap in cleaner fish welfare assessments. By identifying whether the feeding method provided during rearing encourages behaviours that enhance delousing efficiency, optimal husbandry methods can be implemented prior to sea cage deployment.

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INVESTIGATION OF THE OPPORTUNITIES PROVIDED BY LIFE CYCLE ASSESSMENT (LCA) IN THE HUNGARIAN AQUACULTURE SECTOR

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Introduction

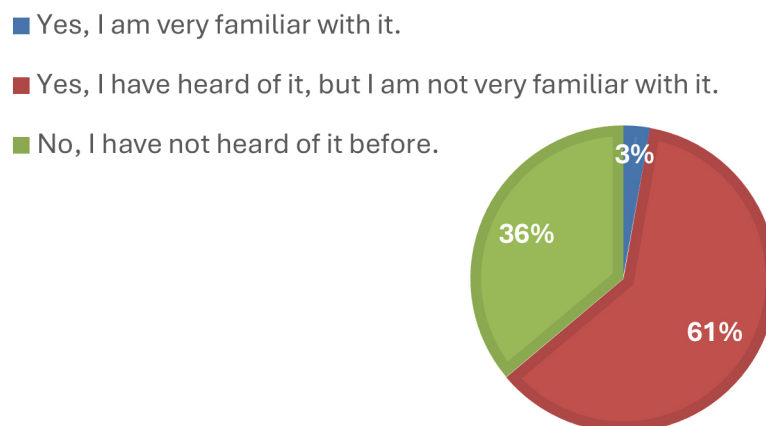
The application of Life Cycle Assessment (LCA) in Hungarian aquaculture is presumed to be limited due to low awareness and uncertain openness among producers, despite its strong potential to improve environmental sustainability. Aquaculture is one of the fastest-growing food production sectors, but it is associated with significant environmental impacts, particularly in feed production, water use, and by-product management (Aubin 2013, Aubin et al. 2015). LCA provides a comprehensive framework to evaluate these impacts across the entire life cycle (Guinée 2002). However, its practical implementation in Hungarian fish farming remains unclear. This study aims to assess the level of awareness, application, and potential of LCA in the Hungarian aquaculture sector.

Materials and methods

The study was based on a combination of literature review and a questionnaire survey conducted among Hungarian fish farms. The questionnaire focused on production characteristics, sustainability practices, feed selection criteria, by-product management, and the awareness and use of LCA. Responses were analyzed to identify trends, challenges, and opportunities related to environmental assessment practices.

Results

The results indicate that although many producers are aware of LCA, its practical application in Hungarian aquaculture remains limited, with most respondents not using any environmental assessment methods. Despite this, sustainability is considered important, and there is clear interest in tools supporting more sustainable decision-making (Bohnes & Laurent, 2019). The main barriers include limited knowledge, lack of accessible tools, and economic constraints, along with challenges related to data collection and methodological complexity. At the same time, positive trends are emerging, such as improved feed management, better by-product utilization, and growing awareness of alternative feed sources (e.g., insect-based proteins) (Seghetta et al., 2017). These findings suggest increasing openness toward sustainability-oriented innovations, despite the current low level of LCA implementation (Figure 1).



1. **Figure:** Awareness of the LCA Methodology Among Farmers

(Continued on next page)

Discussion

The findings suggest that wider adoption of LCA could significantly contribute to reducing the environmental footprint and improving the sustainability of Hungarian aquaculture. However, achieving this requires the development of simplified tools, enhanced knowledge transfer, and stronger economic incentives for producers.

Acknowledgment

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PROTEOMICS OF *Sparus aurata* SEMINAL PLASMA

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Introduction

Seminal plasma provides an optimal environment for the sperm cells to be stored. It is also involved in sperm fertilising ability, motility and optimal metabolic function. This study aimed to assess the proteomic composition of seminal plasma from adult gilthead seabream fish (*Sparus aurata*), a species of high commercial value for European aquaculture.

Material and methods

The present study included three adult male specimens. Milts were obtained during the reproductive season (from October to January) under anaesthesia with 2-phenoxyethanol at a concentration of 0.3 mL/L of water. The ejaculates were centrifuged twice at 4 °C to obtain the seminal plasma. The first centrifugation was performed at 3,000 x g for 20 minutes, and the second at 10,000 x g for 10 minutes. Proteins were identified through an LC-MS/MS analysis.

Results

A total of 1347, 1061, and 1260 proteins were identified in samples from fish 1, 2, and 3, respectively, with 699 common proteins in all samples. Functional enrichment and Gene Ontology (GO) analyses indicated that cathepsin D was significantly represented in the seminal plasma of all fish. The most prominent metabolic function GO term was endopeptidase activity (GO:0004175). Regarding biological processes, the most enriched terms were proteolysis (GO:0006508) and humoral immune response (GO:0006959). Finally, the most enriched GO terms for cellular components were extracellular region (GO:0005576) and proteasome complex (GO:0000502).

Discussion

Cathepsin D is a proteolytic enzyme involved in oocyte maturation and yolk egg processing, regulation of spermatogenesis, stimulation of sperm motility and removal of immature and damaged spermatozoa in fish (Gwon et al., 2017; Sales et al., 2024). The precise role of cathepsin D during spermatogenesis in fish is yet understudied, but proteomic analysis suggests an involvement in protein degradation and apoptotic processes during sperm maturation.

Acknowledgment

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THE EFFECT OF FLOCCULANT AND COAGULANT APPLICATION FOR END-OF-PIPE TREATMENT OF RAS DRUMFILTER BACKWASH ON BIOGAS YIELD AND AEROBIC SLUDGE STABILISATION

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Introduction

Recirculating aquaculture systems (RAS) are optimised for efficient water usage and minimum discharge. The main output in such systems, besides evaporation, is the backwash water required to wash off retained solids from drum filters. In order to increase the water reuse efficiency further, it is possible to treat the backwash water and reintroduce parts of the liquid fraction back into the RAS. This leads to the generation of thickened sludge that can eventually be used as raw material for a series of biorefinery processes such as biogas production and alike.

A chemical process that is commonly used for such water treatment tasks is the application of flocculants and coagulants. Metal-based flocculants and polymer-based coagulants have been extensively studied for their effectiveness in aquaculture systems (Ebeling et al., 2005, Ebeling et al., 2003, Heiderscheidt et al., 2020). However, within today's circular economy paradigm, it is also of interest how such substances affect any follow-up process that utilises the generated sludge. Prior studies in the field of wastewater treatment found that the choice of flocculant or coagulant affects the performance of biological sludge treatment processes (Cainglet et al., 2023).

Against the outlined background, this study assessed the effect of two flocculants (polyaluminum chloride, iron chloride) and two coagulants (starch-based, chitosan-based) on the biomethane yield and sludge stabilisation performance under aerobic conditions.

Material and Methods

The solid fraction of drumfilter backwash water was collected at a commercial RAS farm () producing African catfish (*Clarias gariepinus*). Sampling took place over six hours during a single day, filtering the backwash water through a net with a mesh size of 300 µm, yielding approximately 10 kg of sludge.

Reaching the laboratory, the sludge was mixed with water to reach a dry matter content of 30 g/L. Eventually, the individual flocculants/coagulants were added at dosages determined in a series of pre-trials, mixed at 200 rpm for 1 min and then at 20 rpm for 30 min. Eventually, the treated sludge was left to settle, the supernatant was removed and the sludge designated for biomethane yield tests frozen and shipped.

Biomethane tests were conducted batch-wise according to standard methods (Drosg et al., 2013). In brief, sludge was inoculated with digestate from a biogas plant at a ratio of 3:7 (sludge – inoculum). 500 mL of the mixture was transferred into a 1 L bottle that was sealed airtight. The emerging biogas was stripped of CO₂ by passing it through a gas scrubber filled with NaOH. The purified CH₄ entered a third bottle filled with water, which was displaced into a measuring cylinder according to the produced volume of CH₄.

A second fraction of the sludge was diluted to a dry matter content of 10 g/L, from which 400 mL was filled into each of three 500 mL reagent bottles per treatment, with a control that was not treated with flocculant/coagulant. All bottles were placed in water baths at 20°C and 30°C and continuously aerated for 21 d. The pH and dissolved oxygen concentration were determined daily. In addition, total organic carbon (TOC), total nitrogen (TN), ammonium (NH₄⁺), nitrite (NO₂⁻), nitrate (NO₃⁻), orthophosphate (PO₄³⁻), calcium, magnesium, and iron concentrations were determined in intervals of three days.

Results and Discussion

The experiments were concluded but data analysis is incomplete at the time of abstract submission. Nevertheless, the outcomes of this study are of relevance for RAS farm managers who consider the application of flocculation/coagulation technology in order to meet discharge levels and use of the resulting sludge for biorefinery, e.g., to partly recover energy from by-products.

(Continued on next page)

Acknowledgements

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LENTIL – A DATABASE WITH PERSISTENT IDENTIFIERS FOR WATERS AROUND THE WORLD

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Introduction

What do we know about our waters? Freshwater ecosystems are among the most intensively studied environmental domains. Just a few years ago, the main problem was to handle the vast amounts of data generated. Now that we have entered the age of Artificial Intelligence, the major obstacle is not data handling any longer, but rather their fragmented nature. Information on lakes, ponds, reservoirs, and other inland water bodies is distributed across monitoring programs, research projects, government agencies, biodiversity repositories, and remote sensing products. Some of this data is available in digitalized form, for instance as datasets deposited in data repositories, or in databases via application programming interfaces (APIs). However, the lack of a globally unique and persistent identifier for individual water bodies remains an obstacle to data integration. Meanwhile, differences in names, languages, local identifiers, and other factors hinder the discovery, linking, and reuse of data. This issue affects aquaculture in a similar manner as it affects other research domains and stakeholders outside academia. To overcome this challenge, LENTIL (LENTic InLand waters) provides an open, freely available spatial database that enables persistent identification with the aim of establishing a common reference framework for linking environmental observations, research datasets, and monitoring records to specific water bodies.

Methodology

The starting point of LENTIL is based on a computational pipeline to extract water bodies from the global OpenStreetMap (OSM) database (planet.osm) using the Osmium command-line tool and filtering strictly for standing water classifications (e.g., lakes, reservoirs, basins, and ponds). To create an interactive tool that is capable of bearing the computational load of planetary-scale data, a PostgreSQL database with the PostGIS spatial extension was created. The waterbody ID (wID), a persistent identifier, was then generated and assigned to each waterbody. A change-history table was implemented to track alterations in the underlying OSM dataset and make data still traceable, even though the designated waterbody might have been changed (e.g., split or merged). A user-friendly RShiny app was developed as frontend, which is accessible via <https://waterlentic.org>. The current version of this user interface allows a search for specific water bodies according to their location, name, and wID. Current plans comprise the development of an API and merging of first datasets. Code and data contributions are possible via <https://github.com/TellAnAx/LENTIL>.

Application and Impact

LENTIL provides a foundational infrastructure for freshwater research and management, analogous to the role of DOIs for publications or ORCID identifiers for researchers. By assigning persistent identifiers to freshwater bodies, LENTIL enables the integration of otherwise disconnected datasets. Water chemistry measurements, biodiversity observations, eDNA records, hydrological monitoring data, remote sensing products, ecological assessments, and scientific publications can all reference the same waterbody through its wID. This approach supports the FAIR principles (Findable, Accessible, Interoperable, and Reusable) by providing a stable entity to which data can be linked. Researchers can discover all available information associated with a water body without the need to deal with inconsistent naming (e.g., different names in different languages; multiple waters with the same name) or GIS and coordinate systems.

As environmental monitoring programs expand and data volumes continue to grow, LENTIL offers a scalable solution for improving data integration, reproducibility, and long-term stewardship of freshwater information. The platform represents an important step toward harmonized freshwater data infrastructures and future digital twins of inland water ecosystems. As of now, this tool provides a reliable open-source foundation for planetary-scale analyses of aquaculture infrastructure and water resource management without the need for knowledge in GIS.

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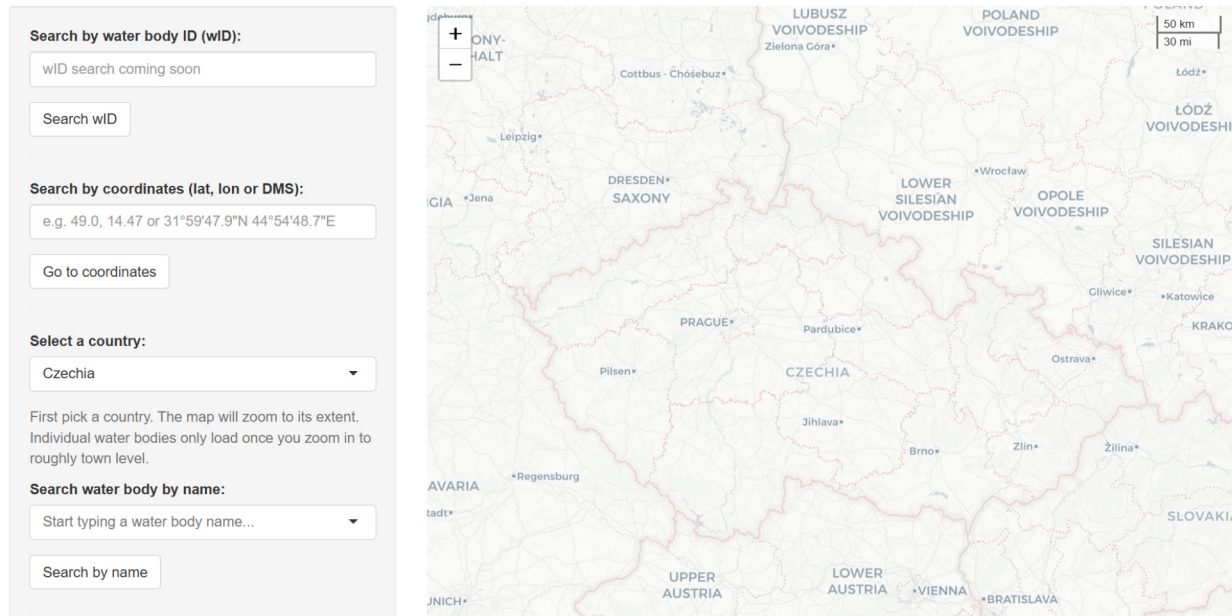


Figure 1: Screenshot of the public user interface available via <https://waterlenti.org>.

Acknowledgements

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FUNCTIONAL FEEDS BEYOND GROWTH: ALGAE MODULATE REDOX STATUS IN SENEGALESE SOLE LARVAE

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Introduction

Early larval stages in marine fish are characterised by rapid growth, high metabolic demand, and still-developing antioxidant systems, making them particularly sensitive to oxidative imbalance. Excess production of reactive oxygen species during this phase can impair cellular function and developmental processes. While nutritional strategies in hatcheries have traditionally focused on maximising growth, there is increasing recognition that growth alone does not fully reflect larval quality. Enhancing physiological robustness, particularly through modulation of redox homeostasis, is emerging as a complementary objective in larval nutrition.

The Senegalese sole is a well-established species in marine aquaculture and a relevant model for nutritional studies during early development, where assessing physiological responses alongside growth can provide a more comprehensive evaluation of larval condition.

Algae-derived ingredients are promising functional feed components due to their richness in bioactive compounds, including pigments, vitamins, and complex sulphated polysaccharides with antioxidant properties. Microalgae such as *Euglena* spp. and *Tetraselmis* spp., together with macroalgae like *Ulva* spp., have been associated with improved physiological responses in aquatic organisms. However, their role in modulating oxidative status during early developmental stages remains insufficiently characterised.

Within this context, this study evaluated the effects of dietary inclusion of algae biomasses on growth performance and oxidative status in Senegalese sole post-larvae.

Materials and Methods

Senegalese sole post-larvae (initial dry weight: 6.6 mg) were reared in a recirculating aquaculture system using 8.8 L flat-bottom tanks, with three replicate tanks per dietary treatment. Water quality conditions were maintained throughout the experimental period, with dissolved oxygen above 96%, temperature at 20.4 °C, and ammonia and nitrite concentrations below 0.1 mg L⁻¹.

From 30 to 43 days after hatching (DAH), larvae were fed one of three experimental diets: a control diet (CTRL), the control supplemented with *Euglena* sp. biomass (EUG), and a diet containing a blend of macroalgae (*Ulva* sp.) and microalgae (*Euglena* sp. and *Tetraselmis* sp.) (MIX).

At 43 DAH, larvae were sampled to assess growth performance and oxidative status. Growth was evaluated through performance indicators including final dry weight (DW), total length (TL), condition factor (K), and relative growth rate (RGR). Oxidative damage was assessed via lipid peroxidation (LPO), while antioxidant responses were evaluated by measuring superoxide dismutase (SOD), catalase (CAT), and glutathione redox balance (GSH:GSSG ratio).

Results and Discussion

At 43 DAH, no significant differences were observed among dietary treatments in growth performance. Average final dry weight reached 22.55 ± 0.45 mg and relative growth rate averaged 10% day⁻¹, indicating that algae inclusion did not influence larval growth.

Despite similar performance, clear differences were detected in larval oxidative status. Lipid peroxidation (LPO) remained unchanged across treatments, indicating no increase in oxidative damage. However, a significantly elevated GSH:GSSG ratio in both algae-supplemented groups points to a more robust redox status, driven by the high availability of reduced glutathione (GSH). This suggests that these diets primed the larvae with a more favourable redox environment, potentially increasing their capacity to mitigate oxidative challenge.

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Superoxide dismutase (SOD) activity was significantly lower in the EUG group and intermediate in the MIX group relative to CTRL, while catalase (CAT) activity remained unchanged. The reduction in SOD activity, coupled with stable LPO levels, suggests a decreased requirement for enzymatic antioxidant defences rather than impaired capacity, indicating a 'sparing effect' facilitated by the non-enzymatic glutathione system. The intermediate response in the MIX group, suggests a co-regulation of antioxidant systems, where the shift is maintained but less pronounced than with *Euglena* alone.

These findings demonstrate that algae-derived ingredients modulate redox homeostasis without inducing oxidative stress or affecting growth performance, supporting their role as functional dietary components during early development.

Conclusion

Algae-based diets modulate oxidative status in Senegalese sole larvae without compromising growth, highlighting their potential as functional feeds targeting physiological robustness beyond conventional performance metrics.

Acknowledgements

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DIETARY PEPTIDE MOLECULAR WEIGHT PROFILE MODULATES INTESTINAL GENE EXPRESSION IN SENEGALESE SOLE LARVAE

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Introduction

The early life stages of marine fish are characterised by rapid growth and profound morphological and physiological changes. In Senegalese sole (*Solea senegalensis*), larval development involves a marked metamorphosis from a pelagic, bilaterally symmetric larva to a benthic post-larval stage, accompanied by extensive digestive tract reorganisation and high metabolic demands. During this critical developmental window, both the immature digestive system and the elevated production of reactive oxygen species (ROS), increase larval susceptibility to oxidative stress, potentially compromising digestive development, nutrient utilisation and overall robustness.

Dietary protein hydrolysates are widely incorporated into larval microdiets due to their high digestibility and the presence of bioactive peptides, which have been shown to enhance digestive function, intestinal maturation, antioxidant capacity and nutrient utilisation in marine fish larvae. In addition to the level of hydrolysate inclusion, the physiological effects of these ingredients are also likely to depend on their peptide molecular weight profile, as peptide weight influences their digestion, intestinal uptake and subsequent physiological responses (Richard et al., 2015; Canada et al., 2017). However, despite increasing evidence supporting the use of protein hydrolysates in larval nutrition, the role of peptide molecular weight profile has received comparatively little attention. Therefore, this study evaluated whether diets containing identical levels of peptide hydrolysates but differing in peptide molecular weight profile modulate growth performance, oxidative status and intestinal molecular responses in Senegalese sole larvae.

Materials and Methods

Senegalese sole larvae were fed from 2 to 17 days after hatching (DAH) with one of two experimental microdiets containing the same inclusion level of peptide hydrolysates but differing in peptide molecular weight profile: a lower-molecular-weight peptide profile (LMP) or an intermediate-molecular-weight peptide profile (IMP). Diets were isonitrogenous and isolipidic and randomly assigned to triplicate tanks.

At 17 DAH, key performance indicators were evaluated through dry weight (DW), total length (TL), condition factor (K), relative growth rate (RGR) and survival rate. Oxidative status was assessed by measuring superoxide dismutase (SOD) and catalase (CAT) activities, and the reduced-to-oxidised glutathione ratio (GSH:GSSG), as well as lipid peroxidation (LPO). Gene expression analysis included antioxidant defence (*sod2*, *cat*, *gpx4* and *nrf2*), digestive enzymes (*ialp*, *ampn*, *lap3*, *tryp* and *ctrb*), appetite and digestive regulation (*cck* and *npv*), nutrient transporters (*pept1*, *lat1* and *fabp2*), and intestinal barrier markers (*cldn15*, *tjp1* and *muc2*).

Results and Discussion

At 17 DAH, larvae reached an average dry weight of 0.67 mg, total length of 7.15 mm, and condition factor of 0.92. Mean survival was 48%, while the relative growth rate averaged 26.8% day⁻¹. None of these performance parameters differed between dietary treatments, indicating that both peptide molecular weight profiles similarly supported early larval growth and survival. Likewise, no dietary differences were observed in SOD or CAT activities, the GSH:GSSG ratio, or LPO, indicating that peptide molecular weight profile did not alter basal redox status.

Despite the absence of phenotypic differences, the peptide molecular weight profile significantly influenced intestinal gene expression. Larvae fed the LMP diet exhibited higher expression of the antioxidant-related genes *cat*, *gpx4* and *nrf2*, suggesting enhanced transcriptional regulation of endogenous antioxidant defences. In parallel, the coordinated upregulation of *lap3*, *cck*, *pept1* and *fabp2* suggests enhanced digestive function and nutrient assimilation, while increased *muc2* expression indicates improved intestinal mucosal protection. In contrast, the expression of *sod2*, *ialp*, *ampn*, *tryp*, *ctrb*, *npv*, *lat1*, *cldn15* and *tjp1* remained unaffected by dietary treatment.

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The coordinated modulation of genes involved in antioxidant regulation, digestion, nutrient transport and mucosal protection suggests that dietary peptide molecular weight profile influences intestinal physiological programming during early ontogeny. Although these transcriptional responses were not reflected in growth performance or redox status at this developmental stage, they indicate that a lower-molecular-weight peptide profile may promote greater physiological readiness of the larval intestine during a period of intense developmental plasticity.

Conclusions

Dietary peptide molecular weight profile modulated the intestinal transcriptional response of Senegalese sole larvae without affecting growth performance or basal oxidative status. A lower-molecular-weight peptide profile induced coordinated upregulation of genes associated with antioxidant defence, digestion, nutrient absorption and mucosal protection, highlighting peptide molecular weight distribution as an important nutritional characteristic to consider when formulating larval microdiets.

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INTEGRATING FAECAL GLUCOCORTICOID PROFILES AND FIN DAMAGE FOR WELFARE ASSESSMENT IN TRIPLOID RAINBOW TROUT

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Introduction

Reliable assessment of fish welfare requires indicators that can be applied under commercial production conditions and capture responses to a range of environmental and management factors. External morphological damage provides practical on-farm welfare information in rainbow trout (Weirup et al., 2022; Toomey et al., 2024), but visible lesions may not fully reflect physiological stress responses. Physiological measures may therefore provide additional information for multidimensional welfare assessment. Faecal glucocorticoid metabolite analysis represents a less invasive alternative to blood cortisol assessment and may offer a delayed and extended window for evaluating physiological stress responses (Ding et al., 2023; Cao et al., 2024). However, relationships between faecal glucocorticoid concentrations and externally observable welfare indicators remain poorly understood, particularly at the individual-fish level. The aim of this study was therefore to evaluate temporal variation in faecal glucocorticoids and fin damage in triploid rainbow trout (*Oncorhynchus mykiss*) and to determine whether individual fin damage was associated with faecal glucocorticoid concentrations after accounting for sampling occasion.

Materials and Methods

The study was conducted within the Interreg Italy–Slovenia CIRCULAR RAINBOW project in a partial recirculating aquaculture system at the Zalog fish farm, Slovenia. Triploid rainbow trout were monitored from stocking in February 2025, when fish weighed approximately 500 g, until March 2026, when they reached approximately 3 kg. Sampling was performed at stocking, approximately one month later, and thereafter at approximately three-month intervals. Fish were anaesthetised with MS-222 before biometric measurements, external welfare assessment and faecal sampling. Fin damage was assessed in 30–60 fish per sampling occasion using an ordinal four-point scale (0–3; 0 = no damage, 3 = severe damage) for six fins. Temporal changes were analysed using an ordinal generalised linear mixed model with a cumulative logit link, including sampling occasion, fin type and their interaction as fixed effects and fish as a random effect. Faecal samples were collected by abdominal massage on five sampling occasions (23–30 fish per occasion), and cortisol, cortisone and tetrahydrocortisone concentrations were determined by liquid chromatography coupled with tandem mass spectrometry. Differences in glucocorticoid concentrations among sampling occasions were analysed using Kruskal–Wallis tests followed by Dwass–Steel–Critchlow–Fligner multiple comparisons. For individual-level associations with glucocorticoids, a composite fin-damage score (FinSum; range 0–18) was calculated as the sum of scores for the six fins. Associations were assessed using partial Spearman correlations adjusted for sampling occasion. As a sensitivity analysis, separate linear models of log-transformed hormone concentrations were fitted with FinSum and sampling occasion as explanatory variables and heteroskedasticity-robust standard errors. Complete paired hormone and fin-damage data were available for 107 fish.

Results

Faecal concentrations of all three glucocorticoid-related compounds differed significantly among sampling occasions (cortisol: $H = 42.26$; cortisone: $H = 56.18$; tetrahydrocortisone: $H = 28.21$; all $P < 0.0001$). The highest median concentrations were observed at the first hormonal sampling in March 2025: 20.28 ng g⁻¹ for cortisol, 281.04 ng g⁻¹ for cortisone and 662.82 ng g⁻¹ for tetrahydrocortisone. Concentrations subsequently remained markedly lower, although temporal patterns differed somewhat among compounds. Cortisol and cortisone concentrations at the first sampling were significantly higher than at all subsequent occasions, whereas for tetrahydrocortisone the difference between March and June 2025 was not significant after adjustment for multiple comparisons. Tetrahydrocortisone was consistently present at substantially higher concentrations than cortisol and cortisone.

Fin condition also varied significantly over time, but the pattern depended on fin type. The ordinal mixed model showed significant effects of sampling occasion ($F_{5,1518} = 19.71$, $P < 0.0001$), fin type ($F_{5,1518} = 54.19$, $P < 0.0001$) and their interaction ($F_{25,1518} = 2.55$, $P < 0.0001$). Fin condition was generally less favourable during the initial sampling, particularly for the dorsal and pectoral fins, and subsequently improved for several fin types; however, temporal changes were not

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uniform or unidirectional across all fins. After adjustment for sampling occasion, individual FinSum was not associated with any of the three glucocorticoids. Partial Spearman correlation coefficients were -0.008 for cortisol ($P = 0.934$), -0.071 for cortisone ($P = 0.478$) and -0.038 for tetrahydrocortisone ($P = 0.702$). Regression models with robust standard errors confirmed the same conclusion (all $P \geq 0.50$).

Discussion

Both faecal glucocorticoid profiles and fin condition showed marked temporal variation, but no detectable individual-level association remained after accounting for sampling occasion. The higher glucocorticoid concentrations observed at the first hormonal sampling, followed by generally lower values, are compatible with temporal changes in physiological state; however, the observational design does not allow these changes to be attributed specifically to adaptation or any other specific cause. The absence of a detectable individual-level association suggests that external morphological indicators and faecal glucocorticoid profiles may reflect different aspects or timescales of the welfare response. Fin damage may represent accumulated physical condition, whereas faecal glucocorticoid concentrations may reflect more dynamic physiological processes. The absence of a concurrent association should therefore not be interpreted as evidence that fin damage is unrelated to welfare or physiological stress. The consistently high tetrahydrocortisone concentrations are noteworthy, particularly as 5β -tetrahydrocortisone has been identified as a prominent cortisol metabolite in Atlantic salmon faeces (Fanjara et al., 2023). Faecal glucocorticoid profiling therefore appears promising as a less invasive physiological component of multidimensional welfare assessment, but further species-specific validation and longitudinal studies are required before reference ranges or routine welfare thresholds can be established.

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DIETARY REQUIREMENTS OF YEARLING COMMON CARP (*Cyprinus carpio*) IN LOW-TEMPERATURE ENVIRONMENTS

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Introduction

In Central European aquaculture, carp shows active and inactive periods depending on weather conditions of the winter season, however as a result of its activity, it often loses weight (Lukowicz & Gerstner, 1998). Feeding occurs from around 6°C and increases with rising temperatures (Terhes, 2025; Chen Song-bo et al., 2012). During milder winter periods, controlled feeding may help maintain body condition and reduce weight loss, supporting healthier stocks till spring. Accordingly, we aimed to determine the requirement for digestible energy and protein for juvenile fish in winter feeding period.

Materials and Methods

Four diets for carp yearlings were formulated and produced by Garant Ltd., Austria. The diets differed in their digestible protein and digestible energy ratios (DP/DE (g/MJ): Diet 1: 18.6; Diet 2: 15.4; Diet 3: 13.8; Diet 4: 11.4). Winter wheat was included as a negative control diet, as it is still commonly used by farmers. The feeding trial lasted over 55 days in three replicate tanks in RAS with initial body weight 99.5 ± 13.8 g and with stocking density 35 fish/m³. Based on previous results 1% of feeding rate (per biomass) was set at constant 10°C. At the end of the feeding trial fish were individually weighed to determine the final body weights in each tank. In addition, Fulton index (condition factor-CF); hepatosomatic index (HIS); viscerosomatic index (VSI); intraperitoneal fat index (IPI) was calculated. The trial continued with the remaining fish population at 20 °C temperature. The stocking density was 19 fish/m³ (~2.2 kg/m³). All the groups were fed with Diet 1 in the feeding rate 3% of biomass. At the end of this 43 days trial growth performance and nutrient utilization parameters were assessed.

Results and Discussion

During the low temperature (10°C) period, the fish consumed and utilized the formulated diets. Body weight increased by 22–27% across treatments, except for Diet 5 (wheat), where only a 5.67% growth was observed. The feed conversion ratio ranged between 1.97 and 2.37, again with the exception of Diet 5, which showed a markedly higher value (10.3). According to the production and nutrient utilization parameters evaluated, Diet 4 showed the best overall performance. However, differences among treatments were not statistically significant for most parameters. The diet with the highest digestible protein-to-digestible energy ratio (Diet 1), performed the worst and exhibited a significantly lower protein efficiency ratio compared to Diet 4. Based on these findings, it seems lower protein and higher energy content is preferable, with an optimal digestible protein-to-digestible energy ratio of approximately 11.44 MJ/kg.

Following the trial mimicking an overwintering feeding regime, the fish were subsequently fed with standard diet (Diet 1), at constants 20 °C temperature. During this period, the fish that had previously been fed wheat (Diet 5) exhibited rapid and intensive growth, which resulted in performance comparable to the other treatments in terms of SGR, FCR, PER, and LER. It appears that the growth potential of this group was also high at 20 °C, compensating the week growth performance in the overwintering period. When comparing the performance of the fish fed formulated diets, significant difference in nutrient utilization was observed between Diet 3 and Diet 4 at 10°C, Diet 4 performed best.

Conclusions

Overall, the performance of the formulated diets was considered highly acceptable and differed significantly from the treatment in which the traditional feeding method was applied. Furthermore, it was demonstrated that lower protein and higher energy content is preferable, with an optimal digestible protein-to-digestible energy ratio of approximately 11.44 MJ/kg.

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BIODIVERSITY AND FISH FARMING IN PONDS: WHAT LESSONS CAN BE LEARNED FROM NATURE-BASED SOLUTIONS?

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Introduction

Fish ponds represent the interweaving of an ecosystem and a socio-economic system, which is managed by humans to support nature's contributions to people (NCPs). These agroecosystems are mainly a heritage of the past, in which land unsuitable for cultivation was transformed into fish-production sites, which can also serve as reservoirs for watering livestock or irrigating crops (Rougier de la Bergerie, 1819). Thus, from the beginning, ponds were created to provide goods and services.

Today, a wide variety of social groups have a range of expectations about and uses for ponds, which serve three categories of purposes (Díaz et al., 2018): non-material or cultural NCPs (e.g., learning and inspiration), regulating NCPs (e.g., regulation of water quantity, regulation of the climate) and material NCPs (e.g., provision of food and feed). It has been shown that not all NCPs are compatible with one another. For instance, food provision conflicts the most with the other NCPs, such as creation of habitat for biodiversity and regulation of water quality and quantity (Bartrons et al., 2024). As ponds help address societal challenges such as meeting demand for fish, one potential approach is to promote nature-based solutions (NbS) (IUCN, 2020). Based on agroecological principles and considering biodiversity as both a driver and a goal, NbS provide an inclusive governance framework built on a culture of compromise. With this in mind, field experiments were performed in 10 fish ponds in France (FEAMP SEPURE project, 2020–2023).

Materials and methods

Researchers and pond fish farmers developed new pond-management strategies together that were designed to make the ponds more economically and environmentally efficient. Management scenarios were based mainly on selecting fish species (planned biodiversity) of economic interest, as a function of their compatibility and complementarity in exploiting resources (Lecocq et al., 2024). Ten scenarios were selected and applied over two consecutive annual production cycles to 10 ponds in three major fish-production regions in France (i.e., Dombes, Brenne and Lorraine).

Biophysical, productivity and environmental analyses were then performed that considered nine NCPs divided into the three NCP categories: Fish production, Fertiliser sources (material NCPs), Climate regulation, Water-quantity regulation, Nutrient retention (regulating NCPs), Creation of habitat for biodiversity, Primary production and Abundance of macroinvertebrates and macrophytes (non-material NCPs).

Results and discussion

The diversity of NCPs identified demonstrated the multifunctionality of ponds. Across all scenarios, most NCPs were regulating NCPs. The functioning of ponds is closely related to their overall biodiversity (both planned and associated). Pond productivity increases when planned biodiversity (fish species assemblages) contains more species and age cohorts. High associated biodiversity supports a high-quality environment and biological productivity. Furthermore, the field experiments in the SEPURE project showed that attention to pond management when stocking fish (i.e., the species, age cohorts, and proportions of each species selected) is essential to ensuring that biodiversity and production do not conflict.

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Acknowledgments

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ValoSEPURE, A PROJECT FOR COMMUNICATION ABOUT THE MANAGEMENT AND DEVELOPMENT OF FISH PONDS IN FRANCE

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Introduction

The FEAMPA project ValoSEPURE aims to develop original and diverse communication initiatives around pond agroecosystems, targeting a broad audience and pursuing several objectives: i) Promoting sustainable and competitive aquaculture practices, ii) Strengthening relations and knowledge sharing among fish farmers at the national level, and iii) Developing a wider community of stakeholders involved in ponds (e.g., aquatic environment managers, aquaculture industry representatives, education establishments and their students).

Materials and methods

The overall goal is to share and enhance knowledge of how fish ponds function and how they are managed, taking into account the new challenges faced fish farmers, with the ambition of collectively identifying new production methods and facilitating dialogue among stakeholders. To this end, the work will build on the advances made during the previous FEAMP SEPURE (New strategies for establishing and managing pond production systems for sustainable fish farming). This earlier project tested several fish-stocking scenarios developed in collaboration with fish farmers and implemented over two years in ten ponds across three aquaculture regions in France (Auvergne Rhône Alpes, Grand Est, Centre-Val de Loire). The main objectives was to improve overall productivity of ponds by revisiting the combinations of fish species, while strictly respecting environment and biodiversity and ensuring a fair income for fish farmers. The project yielded encouraging results, which we aim to disseminate through an ambitious communication plan. In line with the national *Aquaculture Plan for the Future*, this strategy seeks to improve the competitiveness of pond fish farming, promote its development, encourage innovation and even facilitate the establishment of new pond fish farmers.

The communication plan includes the publication of scientific articles and a book in French on the emerging challenges and management of fish ponds. Videos will also be produced to collect and share experiences and feedback from fish farmers and researchers. The development of the *Traits OF Fish* database (<https://toff-project.univ-lorraine.fr>), a tool to support the selection of species combinations in ponds, is another key component of the project, along with the organisation of technical workshops and conferences/debates on pond systems in France.

Conclusion

ValoSEPURE ultimately aims to lay the foundations for creating a renewed reflection on future trajectories for pond systems, including through using Nature-Based Solutions (IUCN). The project seeks to support fish production by relying on the ecosystem services of ponds along with actions to manage, conserve and even restore these systems. All the information needed to participate in knowledge-sharing and dissemination activities will be communicated during the Fish Farming Research Days.

Acknowledgments

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HARMONISED MODULAR LIFE CYCLE ASSESSMENT FOR AQUACULTURE

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Introduction

Life cycle assessment of aquaculture and blue biomass systems is challenged by differences in species, technologies, system boundaries, data structures and modelling assumptions. Primary life cycle inventory (LCI) data are often collected using case-specific spreadsheets, limiting comparability, transparency and reuse. This study presents the design and ongoing development of a harmonised modular framework linking web-based LCI data collection with reusable life cycle assessment (LCA) unit processes.

Methodology

An existing web-based LCI data collection system, already populated with harmonised primary data from aquaculture and blue biomass applications, is being further developed and expanded using a common data architecture applicable to offshore, nearshore and land-based aquaculture. Configurable modules cover hatchery or seed production, feed where relevant, infrastructure, energy and fuels, vessel operations, water and treatment, materials, emissions, cultivation, harvesting, stabilisation, transport, downstream processing, product formulation, packaging, co-products, waste streams and food preparation.

Inventory variables are being standardised using harmonised identifiers, units and metadata and mapped to a reusable unit-process library within a Brightway-based modelling environment. The library can incorporate validated unit processes transferred from existing SimaPro models and informed by documented unit processes from published LCA studies, enabling previous modelling work to be reused across new aquaculture cases. The modular structure allows a given infrastructure configuration to be combined with alternative site-specific productivity and yield data, enabling assessment of the same production technology under different environmental and operational conditions. The architecture is designed to enable case-specific product systems to be assembled according to species, technology, location and value-chain configuration. Mono- and co-cultivation systems can be represented, including shared infrastructure and operations, and system boundaries may extend from primary production to downstream food preparation.

Results and Discussion

The framework provides a common structure for representing substantially different aquaculture systems while maintaining consistent inventory definitions and modelling principles. Common infrastructure and process modules can be reused across systems and combined with site-specific productivity, yield and operational data, allowing the influence of location and production performance to be assessed independently of infrastructure design. Technology- and species-specific modules can be activated as required. For co-cultivation, shared activities can be distinguished from species-specific inputs and outputs, supporting transparent modelling of operational synergies.

The framework builds on harmonised LCI data already collected through existing studies (e.g. e.g., Chakraborty et al., 2026; Pandey et al., 2026; Sainz de la Maza Larrea et al., 2026). Ongoing development extends coverage to additional production technologies, sites and downstream processes and establishes traceable links between the harmonised LCI data and reusable unit processes. The generic architecture also accommodates land-based aquaculture systems. This provides the basis for automated construction and calculation of case-specific LCA models.

Conclusion

A harmonised web-based LCI infrastructure combined with reusable unit-process models offers a scalable approach for transparent, reproducible and comparable LCA across aquaculture and blue biomass systems. The framework is intended to reduce repeated manual model construction while retaining the flexibility required to represent different species, technologies, production environments and system boundaries from aquatic biomass production to food products.

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OUTSOURCING IN AQUACULTURE: A MIXED-METHODS STUDY OF INTERORGANIZATIONAL COLLABORATION AND SAFETY

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Introduction

Norwegian salmon farming companies rely heavily on specialized contractors in their sea-based operations, including well boats, slaughter vessels and specialized service vessels. To ensure health and safety of all personnel in these operations, a well-functioning collaboration between fish farm employees and contractors is crucial.

Results

Results from an interview study conducted in 2025, and a digital survey conducted in the period February – April 2026 will be presented. First, the interview study included 20 informants from fish farming companies and contractors. Several challenges related to safety and collaboration were identified, including pressure to conduct operations even though personnel safety may be compromised; a lack of planning and communication across organizations; and lack of practical experience and competence.

After the interviews, the survey further explored employees' perceptions of challenges related to the collaboration. As part of the survey, more than 1850 employees working in a) fish farming companies and b) independent ship owners/ service vessel companies (contractors) were presented with safety related statements regarding the quality of internal relationships and the relationships with the other party. A 5-point Likert scale (strongly disagree - strongly agree) was applied, and statements "mirrored" each other. For example, fish farm employees evaluated the statements: "*Our efficiency demands mean that external contractors sometimes have to break the safety procedures*" and "*Lack of cooperation with external contractors is a threat to safety*". Contractors evaluated the statements: "*The fish farming companies' demands for efficiency mean that we sometimes have to break the safety procedures*" and "*Lack of cooperation with the fish farming companies is a threat to safety*".

The interview and survey results provide new knowledge regarding challenges related to collaboration and perceptions of the relationship between fish farming companies and contractors. This is valuable in the search of measures that can improve safety in an accident-prone industry.

Acknowledgment

This research 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 number 902016).

UNLOCKING INNOVATION: A STUDY OF TECHNOLOGY DEVELOPMENT IN NORWEGIAN SALMON FARMING

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Introduction

This presentation addresses innovation in Norwegian salmon farming. With implementation of new technologies pointed out by regulators as crucial to handle prominent challenges in coastal sites, and fish farmers needing optimal solutions for offshore aquaculture operations, the topic is timely. Document analysis and in-depth interviews with experienced people working for suppliers and fish farming companies conducted in 2025-2026 provide insight into key drivers, barriers and success factors for technology development that are relevant to consider moving forward.

Results

Drivers for innovation relate to requirements found in current and expected national regulations pointing towards the need for new technologies. For offshore sites in particular, new farm concepts are crucial to operate safely. Both established and new suppliers are eager to develop what the industry needs to achieve sustainable and profitable production of fish. Fish farmers' internal strategies and decisions regarding the technologies they develop and obtain are consequently essential for the suppliers.

Barriers for technology development in the industry over all include regulatory and framework conditions, economic-, knowledge-related- and relational barriers. Regulations are seen as unpredictable and ill-suited for industry needs. Furthermore, economic barriers include costs, access to capital (investors) and the fish farming companies' willingness to take risks. Procurement capabilities and lack of strategies for innovation are also related to this. Different types of knowledge are often needed for successful innovation processes, and barriers include access to both experience based and experimental knowledge. Suppliers also need access to fish farms to test new solutions in realistic environments. Interviewees described problem-solving in the industry as reactive and pragmatic, applying a trial and failure approach, traditionally occurring within the local communities where the companies are located.

Success factors for innovation for suppliers are still considered to be geographic presence to fish farming companies, as well as access to capital and strong relationships both within and across organizations. A shared understanding among suppliers and fish farmers, and openness from the fish farmers about their challenges to attract entrepreneurs is considered important to unlock innovation moving forward.

Acknowledgment

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A NEW LARVAL IN VITRO MODEL OF RAINBOW TROUT (*Oncorhynchus mykiss*) FOR ECO-PHYSIOLOGICAL AND ECO-TOXICOLOGICAL RESEARCH

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Introduction

The Rainbow trout (*Oncorhynchus mykiss*) is one of the widely farmed fish species worldwide. This makes it to a key model organism in aquaculture research. Therefore, in vitro models are developed in eco-physiological and eco-toxicological research for analysis at the cellular level. In this way, a wide variety of scientific questions can be precisely measured under controlled conditions.

In present study, we are introducing you to our new cell model OMYlar5, derived from larval rainbow trout. With this model, we are able to examine for example, the impact of increasing temperatures or hypoxic conditions on physiological parameters as well as genetic and metabolic alterations. In accordance with the 3Rs principle, cell-based studies can replace or reduce the number of animal-based studies used in ecology and physiology research.

Material and Methods

The cell line OMYlar5 was isolated from one rainbow trout larva and is currently in passage 56. Proliferation of OMYlar5 cells was analysed and compared at 18°C, 20°C, and 22°C to evaluate the optimal growth condition and possible temperature-related effects. All cells were cultivated in Leibovitz-15 Medium (L-15) supplemented with 10% fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin (P/S). Growth and vitality were measured by trypan blue staining and image-based automated cell counting (EVE™ Plus).

Morphological cell changes were visualized by phase-contrast (AE2000 microscope with a Moticam 5 Plus camera) and actin staining with Phalloidin-iFluor 488 Reagent (DM400B fluorescent microscope). Additionally, mRNA abundance of selected temperature- and stress-related genes was measured by quantitative reverse transcription PCR (RT-qPCR). Furthermore, immunofluorescence staining with heat shock markers Hsp70 and Hsp90 (Cell Signaling, cat. #4872 and #4874), expressed during stress conditions, was conducted. The impact of temperature on OMYlar5 mitochondrial function was analyzed by measuring oxygen consumption rates using Seahorse technology (Agilent). Statistical significance was calculated using two-way ANOVA followed by Tukey's post-hoc modification. A p value < 0.05 was considered significant.

Results

Morphological observation and cytoskeletal analysis revealed that confluent cells growth at 18 °C and 20 °C displayed a round, epithelial-like morphology. In contrast, at 22 °C, fibroblast-like cells were present with a mosaic-like arrangement. Cells also tended to form multi-layered structures more frequently. Growth analysis revealed that OMYlar5 cells incubated at 22 °C exhibited the highest proliferation rate, which corresponded with the greatest cell density and the shortest doubling time (not significant). Cell size and viability were not affected by temperature.

Gene expression analysis of stem cell-markers, temperature-related genes, and stress-related genes characterized the larval rainbow trout cell line at the molecular level. Furthermore, we detected immunofluorescence signals of Hsp70 appearing in the nucleus and vesicle-like structures of cells, and Hsp90 showing a uniform distribution within the cytosol in all three temperatures.

In regard to metabolic analysis, we determined mitochondrial oxidative respiration by measuring basal and FCCP-mediated oxygen consumption rates at 18, 20 °C and 22 °C. Comparison of key parameters like basal respiration, mitochondrial ATP production, and maximal respiration allows conclusions regarding temperature-induced metabolic changes in OMYlar5 cells.

Discussion

Aquaculture research, as a rapidly growing sector, requires simple, consistent diagnostic tools to support efficient research. Therefore, in vitro models derived from fish offer a cost-effective and controllable approach for studying environmental variables such as temperature, oxygen saturation or ecotoxic substances.

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In regard to global warming, increasing water temperatures and reduced oxygen levels pose major problems for fish populations as well as aquaculture facilities. There are numerous interesting and important *in vivo* studies on rainbow trout that provide valuable knowledge. These studies could be supplemented or replaced by research using cell-based models. Using the larval cell line OMYlar5, our study demonstrates that increasing temperatures induce measurable changes in cell growth, morphology, gene expression, and metabolism. These results underscore the value of this *in vitro* system for eco-physiological and eco-toxicological research, as well as to develop a deeper understanding of the cell physiology of *O. mykiss*.

Using the OMYlar5 cell line in experimental approaches, aligns with the 3Rs principles by reducing and replacing the use of animals and refining experimental methods in aquaculture research and therefore providing a practical alternative to experiments using live fish.

BEYOND CONVENTIONAL METRICS: INTEGRATING BIOTELEMETRY AND PHYSIOLOGICAL INDICATORS TO ASSESS *Sparus aurata* WELFARE ACROSS CONTRASTING AQUACULTURE SYSTEMS

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Introduction

Fish welfare assessment has become a central concern in marine aquaculture, both for optimising production efficiency and addressing growing consumer awareness. Gilthead seabream (*Sparus aurata*), one of the most commercially significant species in Mediterranean aquaculture, is farmed under a range of systems including conventional and organic regimes, yet science-based welfare evaluation tools grounded in real farming data remain scarce. This study aimed to assess *S. aurata* welfare across contrasting aquaculture environments by combining established physiological indicators with cutting-edge biotelemetric monitoring at two operational sea farms.

Materials and Methods

The investigation was carried out at two offshore cage farms in Sardinia, Italy: a conventional facility A and a certified organic operation B, over an approximately four-month period (August–November 2024). A multidisciplinary framework integrated classical welfare indicators, including growth performance and a suite of stress, immune, and metabolic blood markers (haematological parameters, lactate, glucose, cortisol, IGF-1, total protein), with surgically implanted biotelemetric tags. These tags, incorporating accelerometers and depth sensors, recorded continuous swimming activity and vertical habitat use in a subset of fish over roughly 100 days. Data were analysed through both univariate and multivariate statistical methods.

Results

Substantial inter-site and temporal differences emerged across parameters. Multivariate analysis indicated superior welfare status in fish from Stintino, evidenced by greater growth performance and elevated total protein levels, suggestive of a stronger immunological condition. Despite higher circulating lactate, fish from the B site displayed lower acceleration values, pointing to a reduced reliance on anaerobic pathways relative to fish at A site. Fish from the conventional site appeared more metabolically constrained, potentially limiting their resilience to future stressors. Growth disparities between sites were attributed primarily to age-related differences rather than differential metabolic allocation. Multiple site-specific factors, including environmental conditions, stocking density, feed regimes, and predator interactions near the cages, likely underlie the observed metabolic divergence. Telemetry further revealed clear diel activity patterns and marked inter-site differences in vertical distribution, driven by environmental cues and potential predator presence. Acceleration profiles were also consistent with feeding-anticipatory behaviour, corroborating existing literature.

Conclusion

This study delivers a detailed characterisation of growth, health, and welfare indicators under operational farming conditions, highlighting the complexity inherent in welfare interpretation when multiple parameters yield divergent signals. The multi-indicator framework employed here provides a robust and comprehensive welfare assessment alongside a unique real-world dataset for *S. aurata* reared in open-sea cages. These findings constitute a meaningful baseline for future research aimed at explaining the inter-individual, inter-site, and temporal variability observed in farmed gilthead seabream.

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HOW SEASONAL VARIABILITY SHAPES TISSUE RESPONSES IN TUBERCULATE ABALONE *Haliotis tuberculata* IN THE NORTHERN ADRIATIC SEA

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Introduction

In the Northern Adriatic Sea, populations of tuberculate abalone (green ormer) (*Haliotis tuberculata* L.) are increasingly threatened by bottom trawling and disturbances related to seafood harvesting, leading to a noticeable decline. This region is characterized by a relatively shallow average depth of approximately 30 m and a gentle bathymetric gradient. Key oceanographic factors in the Adriatic include seasonal variations in salinity, nutrient concentrations, light availability, currents, and temperature. Fluctuations in these parameters—particularly temperature, algal dynamics, and wave action—strongly influence the growth and physiological processes of these herbivorous gastropod mollusks. Consequently, tissues of green ormers from the Northern Adriatic were examined across the principal seasonal periods, focusing on their microanatomical features, as well as biochemical characteristics and antioxidant defense mechanisms.

Materials and Methods

A total of 135 wild *H. tuberculata* of both genders were retrieved over yearly seasons to permit the analysis of individuals in various physiological states. The total antioxidative status (TAS), glutathione peroxidase (GPx), and superoxide dismutase (SOD) activities were measured with Randox commercial kits (Dublin, Ireland) on Abbott Architect c4000 (Abbott, Germany) biochemical analyzer. Lipid peroxidation was established according to the formation of thiobarbituric acid reactive substances (TBARS), as a result of the reaction between malondialdehyde (MDA) and TBA. Lipids for determination of fatty acid composition were extracted; For gas chromatographic analysis, 1 µL of prepared methyl esters was injected into the Agilent Technologies 6890N Network GC system (Santa Clara, CA, USA) equipped with flame ionization detector. Fatty acid methyl esters (FAME) were separated on a DB-23 capillary column (60 m×0.25 mm×0.25 µm, Agilent Technologies). Fatty acids were identified by comparing their retention times with the retention times of 37 Component FAME Mix (Supelco, Sigma-Aldrich, St. Louis, Missouri, USA). The surface normalization method was used to determine the quantitative composition of fatty acids expressed as the percentage of total fatty acids.

Results and Discussion

Temperature shifts in terms of seasonal extremes, gradual climate change and discrete harsh climatic events may alter the structure of marine populations and have detrimental effects on gastropod homeostasis. The Northern Adriatic is a hot spot in terms of sea temperature rise in relation to climate change, with consequences such as seawater warming and salinity increasing. Tissue concentrations of glucose (GLU), triglyceride (TRIG) and cholesterol (CHOL) were determined as the main energy sources for metabolic demands. The activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), total antioxidant status (TAS) were evaluated as parameters for antioxidant capacity and lipid peroxidation. Tissue magnesium (Mg) and calcium (Ca) were investigated as formative shell elements, crucial for ionic homeostasis and reproduction control. Significant differences were found between spring/summer and winter samples for TAS, with peaks in June. GPx had the highest activities in fall samples, just as SOD, CHOL, GLU, TRIG, Mg and Ca parameters. Differences in green ormer tissue biochemical and antioxidant defense properties over seasons were evident. Data were grouped tightly in ormer tissues in spring/summer and winter, having unsymmetrical interquartile ranges. Long-term temperatures between 8.5 and 9.0°C are the lethal limit for ormers, and at low temperatures, they are limited in their ability to absorb food and grow. Winter low temperatures thus contributed to lower TAS and SOD concentrations. Not only antioxidant capacity, but also tissue Mg, Ca and GLU were on the decreased levels in ormers in winter. However, in fall a number of parameters had their yearly peaks, such as Mg, Ca, SOD, GPx, TRIG, CHOL and GLU. Interestingly, in this work ormers had elevated total antioxidant status in the warmer period, which can be attributed to the availability of food containing antioxidant compounds. During the spring/summer season, gonads were at the mid-maturation stage, and in fall, the ripe and spawned stages were recorded.

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The fatty acid composition of soft tissues varied between seasons, as well as between genders. Univariately, there was a significant variation between seasons for all fatty acids except C22:5n3, and between genders for all except C21:0, indicating that the main driver for gender differences stems from the interaction effect of season and gender. In particular, the concentrations of C15:0, C17:0, C18:0, C18:2c, C20:4n6, C20:5n3, and C22:5n3 were higher in males than in females during the warmer time of the year and the remainder were higher in females than males. The proportions of total monounsaturated fatty acids (MUFA) increased in autumn. The increase of the total ormer PUFA contents in this study in winter was expected. It was mainly due to higher n-3 PUFA concentrations. DHA is most likely not synthesized by ormer, which can accumulate DHA in body membranes only when DHA is present in its diet. The proportions of total saturated fatty acids (SFA) were similar in spring and autumn, the highest in summer and the lowest in winter.

Overall, the adjustments of wild green ormer body homeostasis to seasonal temperature shifts in the Northern Adriatic were reflected in their lipid peroxidation and antioxidant capacity, proximate and fatty acid tissue composition. Our results highlight these self-regulating processes by which ormer biological systems strive to maintain stability while adapting to diverse temperature/seasonal conditions.

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SOCIAL PERCEPTION OF AQUACULTURE: ANALYSING THE RISE OF ANIMAL WELFARE CONCERNS IN EUROPEAN AQUACULTURE DISCOURSE

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Introduction

Aquaculture is the fastest-growing sector of animal food production worldwide and a key contributor to future food security. However, its expansion in Europe remains slower than expected, partly due to increasing social concerns (Pérez et al., 2025). Beyond regulatory and spatial limitations, public acceptance has emerged as a major constraint. Social perception of aquaculture is shaped by factors such as environmental sustainability, food safety, animal welfare, and trust in governance (Pérez et al., 2025). In recent years, ethical concerns about the treatment and sentience of aquatic organisms have gained prominence. Animal advocacy groups have increasingly targeted aquaculture practices, questioning farming conditions and slaughter methods (Clemente et al., 2023). Understanding the influence of these narratives is crucial for promoting transparent and socially accepted aquaculture systems. This study examines the role of animal welfare advocacy in shaping public attitudes toward aquaculture in Europe and its implications for the sector's future.

Social Perception and Knowledge Gaps in Aquaculture

Public understanding of aquaculture remains limited compared with terrestrial livestock production. Many consumers have only a general awareness of the sector and lack detailed knowledge of production systems, environmental management, and regulatory frameworks (Brenes & Torres, 2025). This limited familiarity means that perceptions are largely shaped by external narratives rather than direct knowledge. Media coverage, non-governmental organizations, and social networks frequently become the main sources of information shaping public opinion about aquaculture. Despite these knowledge gaps, citizens frequently express environmental concerns, including potential contaminant release, excessive veterinary treatments, and interactions between farmed and wild populations. However, more recently, ethical considerations related to animal welfare have begun to occupy a central role in public discourse (Pérez et al., 2025).

Animal Welfare Advocacy and Aquaculture

Animal welfare has become a central issue in contemporary food system debates. Although historically focused on terrestrial livestock, advocacy campaigns have increasingly extended to aquatic organisms. Scientific research over the past two decades has supported this shift, providing evidence that many fish species can perceive noxious stimuli and display responses consistent with pain perception (Orth, 2023). These findings have influenced both public perception and policy discussions. In Europe, surveys indicate that citizens express growing concern for the welfare of animals used in food production, including fish (Savanta ComRes, 2018; European Commission, 2023). However, knowledge of aquaculture systems remains limited, amplifying the influence of advocacy narratives. Welfare organizations commonly highlight issues such as high stocking densities, handling stress, disease management, and slaughter practices, particularly the lack of standardized stunning methods in some systems (Mercogliano et al., 2024). Additionally, discussions on the sentience of cephalopods (e.g., octopuses) and decapod crustaceans (e.g., lobsters) have further expanded ethical concerns in aquaculture.

Influence of Advocacy Narratives on Public Attitudes

Environmental advocacy campaigns often use communication strategies that highlight animal suffering, industrial intensification, and consumer responsibility to raise awareness of ethical concerns in food production. In aquaculture, these narratives can strongly shape public opinion, particularly given the limited transparency and understanding of farming practices. As a result, discourse disseminated through popular channels such as social media may play a key role in constructing the sector's social image (Budhathoki et al., 2024). Nevertheless, these debates have also driven positive change, promoting improved husbandry practices, the development of welfare indicators, and more humane slaughter techniques.

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Implications for the Aquaculture Sector

The increasing visibility of animal welfare debates presents both challenges and opportunities for aquaculture. Negative public perceptions may hinder sector expansion and consumer acceptance, but growing societal concern can also drive innovation and higher production standards. Addressing these challenges requires a proactive approach based on transparency, effective scientific/technical communication, and stakeholder engagement. Integrating welfare considerations into management frameworks may improve both ethical standards and production efficiency. In this context, research on welfare indicators, humane slaughter technologies, and environmental sustainability can help demonstrate the sector's commitment to responsible production and strengthen public trust.

Conclusions

Social acceptance is increasingly recognized as a key factor shaping the future of the European aquaculture sector. Among the drivers of public perception, animal welfare has gained prominence, influenced by advocacy movements and growing scientific evidence on the sentience of aquatic species. Despite limited public understanding of aquaculture systems, ethical concerns about the treatment of farmed aquatic organisms are becoming more visible. This trend underscores the need for improved communication among scientists, producers, policymakers, and society. Addressing welfare issues through evidence-based practices and transparent communication can strengthen the sector's social legitimacy and support the development of sustainable and ethically responsible food systems.

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CAN LAND-BASED CULTURE MATCH NATURAL GROWTH? A COMPARISON OF *Ruditapes decussatus* PERFORMANCE IN INDOOR TANKS AND ESTUARINE BEDS

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Introduction

Since the early twentieth century, intensive harvesting of the grooved carpet shell (*Ruditapes decussatus*) has contributed to a marked decline in natural populations throughout its distribution range. Despite the species' capacity for natural recruitment, the reduction of wild stocks has prompted major producing countries, including Spain, France, and Portugal, to promote aquaculture as a sustainable alternative to support production while reducing harvesting pressure on natural ecosystems. Traditionally, *R. decussatus* has been cultivated in estuarine and intertidal environments, where production is strongly influenced by natural environmental variability, predation, disease outbreaks, and extreme climatic events. In contrast, land-based tank culture offers greater control over environmental conditions and husbandry practices, potentially improving animal management and production stability. However, information comparing the productive performance of clams reared under controlled indoor conditions with that of individuals grown in their natural estuarine habitat remains limited. The present study compared the growth performance of *R. decussatus* cultured in indoor land-based tanks with that of clams reared under natural estuarine conditions (Piedras Piver, El Rompido, Huelva) throughout three months. The results provide valuable information on the feasibility of controlled land-based systems as an alternative or complementary strategy for the cultivation of this economically important bivalve species.

Materials and Methods

R. decussatus juveniles were obtained through thermally induced spawning at the IFAPA Agua del Pino facilities (Cartaya, Huelva, Spain). Before the experiment, a representative sample of clams was collected for initial biometric measurements. Clams were randomly distributed among 20 mesh baskets (300 individuals per basket) and reared for three months, from May (T_0) to August (M3) 2026, under two experimental conditions: (i) 10 baskets deployed in a natural estuarine environment (OUT) and (ii) 10 baskets maintained in controlled indoor tanks (IN). In both culture systems, the substrate depth was maintained at 10 cm and consisted of a mixture of silica sand (95%) and aragonite (CaCO_3 ; 5%), with a grain size of 3–4 mm. Clams reared under natural estuarine conditions fed on naturally available phytoplankton. In contrast, those maintained in indoor tanks were supplied with a mixed microalgal diet predominantly composed of *Skeletonema* spp. and *Chaetoceros* spp. Water quality parameters, including nutrient concentrations, chlorophyll-a concentration, temperature, and dissolved oxygen saturation, were monitored fortnightly throughout the experimental period. Total weight and shell length were assessed monthly by measuring a random subsample of at least 50 clams from each experimental group. At the beginning (T_0) and end of the three-month rearing period (M3), soft tissues were collected from 24 individuals per experimental group (IN vs OUT) to assess growth performance. Subsequently, 12 clams per group were oven-dried at 80 °C and then incinerated at 450 °C for 24 h in a muffle furnace to determine meat dry weight and ash content.

Results and Discussion

Both culture systems supported substantial growth of *R. decussatus* throughout the three-month experimental period (Figure 1). Total weight and shell length increased progressively from T_0 to M3 in both IN and OUT groups, confirming that juveniles were able to adapt to both controlled indoor and natural estuarine conditions. However, significant differences between culture systems became evident during the rearing period, with clams maintained in the estuarine environment generally showing higher weight and length at the end of the experiment.

Although both culture systems resulted in a marked increase in tissue biomass throughout the experimental period, no significant differences were detected between IN and OUT groups for either meat wet or dry weight. In contrast, shell weight was significantly higher in clams reared under estuarine conditions than in those maintained in indoor tanks (Table 1). This pattern suggests that the natural estuarine environment may have provided more favourable conditions for shell deposition and structural growth. Ash weight increased progressively from T_0 to M3, with significant differences among groups. However, relative ash content showed a different pattern, decreasing from 2.27% at T_0 to 1.73% in IN clams, while increasing to 2.56% in the OUT group (Table 1). The higher ash accumulation observed in OUT clams is consistent

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Figure 1. Total weight (A) and shell length (B) of clams reared for three months under two culture conditions: controlled indoor tanks (IN) and a natural estuarine environment (OUT). Data are presented as mean $\pm$ SEM ($n \geq 50$). Different letters (a,b,c) indicate significant differences among sampling times (T0,M1, M2,M3) within the same rearing condition (IN or OUT), whereas an asterisk (*) indicates significant differences between IN and OUT clam groups at the same sampling time

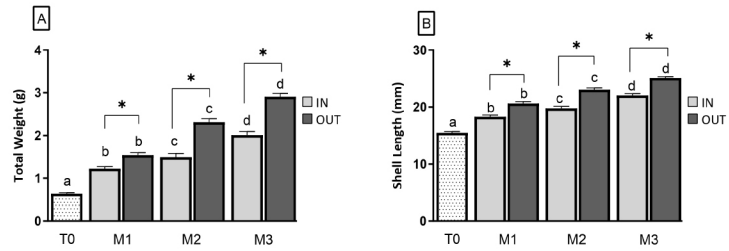


Table 1. Growth parameters of *Ruditapes decussatus* reared for three months (M3) under two culture conditions: controlled indoor tanks (IN) and a natural estuarine environment (OUT). Data are presented as mean $\pm$ SEM ($n = 24^*$ and 12^{**} , respectively). Different letters (a,b,c) indicate significant differences among groups (one-way ANOVA followed by Tukey's post hoc test, $p \leq 0.05$)

	T ₀	IN (M3)	OUT (M3)
Meat Wet Weight (g)*	0.22 $\pm$ 0.01 ^a	0.75 $\pm$ 0.04 ^b	0.85 $\pm$ 0.04 ^b
Meat Dry Weight (g)**	0.03 $\pm$ 0.00 ^a	0.12 $\pm$ 0.01 ^b	0.14 $\pm$ 0.01 ^b
Ash Weight (mg)**	5.00 $\pm$ 0.41 ^a	13.94 $\pm$ 0.90 ^b	23.19 $\pm$ 2.19 ^c
Ash Content (%)**	2.27 $\pm$ 0.03 ^b	1.73 $\pm$ 0.03 ^a	2.56 $\pm$ 0.04 ^c
Shell Weight (g)*	0.31 $\pm$ 0.01 ^a	1.10 $\pm$ 0.06 ^b	1.32 $\pm$ 0.05 ^c
Meat Yield (%)*	33.90 $\pm$ 0.56	33.88 $\pm$ 0.99	32.32 $\pm$ 0.51
Shell Component Index (%)*	48.78 $\pm$ 0.33 ^a	49.64 $\pm$ 0.62 ^{ab}	50.37 $\pm$ 0.47 ^b
Condition Index (Dry Weight)**	9.57 $\pm$ 0.43	9.77 $\pm$ 0.35	10.90 $\pm$ 0.50

with their greater development and may reflect enhanced mineral deposition under natural estuarine conditions. Despite the differences observed in shell development, meat yield remained relatively stable across groups, with no significant differences detected. Likewise, the dry-weight condition index did not differ significantly among groups (Table 1). Overall, both culture systems supported juvenile *R. decussatus* growth over three months. However, estuarine conditions promoted greater shell development and ash accumulation, while indoor culture achieved comparable meat yield and condition, supporting its potential as a complementary strategy for environmental controlled production.

Acknowledgments

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DIETARY MICROENCAPSULATED BOTANICALS AND ORGANIC ACIDS PROMOTES SYSTEMIC THERMAL RESILIENCE IN EUROPEAN SEABASS (*Dicentrarchus labrax*)

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Introduction

Mediterranean aquaculture is being threatened by yearly summer heatwaves phenomena, exposing fish to prolonged thermal stress with negative effects on health, immune function, and performance, especially for temperature-sensitive species (Martínez et al. 2023). Chronic heat stress (HS) in fish triggers a cascade of physiological responses, including upregulation of heat shock proteins and induction of systemic inflammation, ultimately compromising gut integrity, nutrient absorption, and overall health (Ma et al. 2025). Among the nutritional strategies investigated to mitigate HS effects, dietary botanical compounds and organic acids have shown promising results to enhance fish resilience both under homeostatic and stressful conditions (He et al. 2017; Caruso et al. 2026; Busti et al. 2020). This study investigated the systemic effects of a microencapsulated blend of botanicals and organic acids (AviPlus®Aqua) on the molecular stress response of European seabass (*Dicentrarchus labrax*) subjected to chronic HS, with a multi-tissue gene expression approach.

Materials and Methods

A total of 720 European seabass (initial body weight ~10 g) was placed into 12 tanks of 300 L in a marine RAS and allocated into 4 experimental groups (3 tanks/treatment, 60 fish/tank): thermoneutral control (CTR), thermoneutral treated (TRT), heat stress control (CTR-HS), and heat stress treated (TRT-HS). Treated groups received dietary supplementation of AviPlus®Aqua at 2 kg/MT of feed. Fish were acclimated on the study diets for one week at 25°C, after which water temperature was gradually increased by 1°C/day up to 30°C (5 days) and maintained for 23 additional days. The start of the acclimation period was considered as day 0, day 7 is the start of the thermal challenge and day 35 is the end of the challenge. Fish were weighed on d0 and d35 to evaluate growth performance. At the end of the trial, 9 fish per group (n=3 per tank) were sacrificed for qPCR-based gene expression analysis on intestine, liver and gill. A panel of markers related to inflammation (*il-1β*, *tnf-α*, and *il-10*) and stress-adaptation (*hsp70*, *hsp90*, *gr*) was assessed. Relative gene expression of selected markers was normalized using two housekeeping genes (*β-actin* and *ef1α*) following $2^{-\Delta\Delta Ct}$ method. Two-way ANOVA was used to evaluate the effects of dietary treatment and HS and their interaction, with significance set at $p \leq 0.05$, using GraphPad Prism v.11.0.1 (GraphPad Software, Inc., Massachusetts, USA).

Results and Discussion

Growth performance at d35 was significantly affected by both HS and dietary treatment, with treated fish showing consistently better results (+5% of average weight and 0.14%/day of specific growth rate) than controls, particularly under HS. Concerning gene expression (Figure 1), two-way ANOVA revealed a significant effect of thermal challenge across all three target tissues, confirming the systemic molecular impact of chronic HS. Also, a significant treatment × temperature interaction was detected in most of the statistical comparisons across markers and tissues, indicating a stronger supplement effect under HS conditions. In particular, HS significantly upregulated the expression of *il-1β*, *tnf-α*, *hsp70*, *hsp90*, and *gr* (glucocorticoid receptor, a key mediator of cortisol-driven stress response) across the target tissues compared

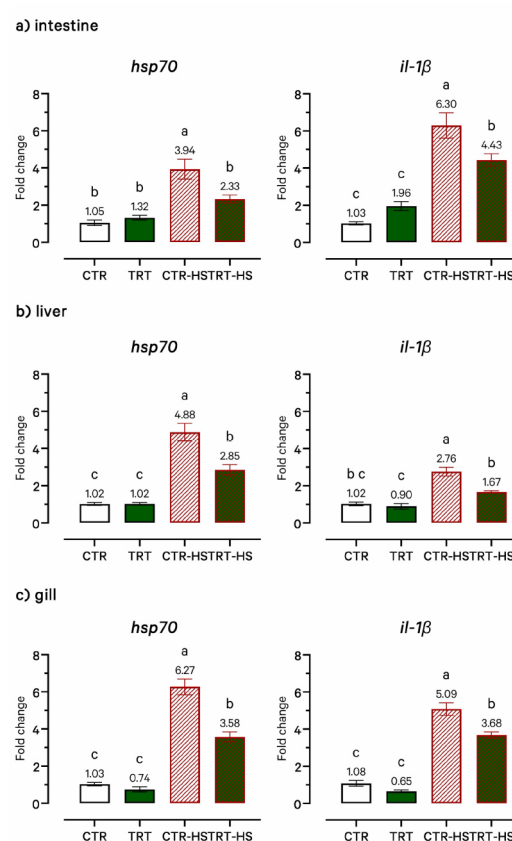


Figure 1. mRNA levels of *hsp70* and *il-1β* in (a) intestine, (b) liver, (c) and gill. Data are means ($n = 5$) $\pm$ SEM as vertical bars. Different letters indicate statistical significance with $p \leq 0.05$.

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to thermoneutral conditions, while *il-10* was reduced. This molecular signature is consistent with a sustained inflammatory and stress response. The dietary treatment attenuated the HS-dependent upregulation of *hsp70*, *il-1 β* , and *tnf- α* in intestine, and *hsp70*, *hsp90*, *gr*, *il-1 β* , and *tnf- α* in both gill and liver in TRT-HS fish compared to CTR-HS, with expression levels being restored to values comparable to those of CTR. Regarding *il-10*, the treatment produced a tissue-specific response, with a significant increase in gill and liver of TRT-HS fish compared to CTR-HS (restoring to value comparable with thermoneutral conditions), while an upregulation was also observed in the intestine, collectively suggesting a promotion of anti-inflammatory tone across all examined tissues.

Conclusions

This multi-tissue approach highlights the potential of targeted nutritional interventions to improve the adaptive capacity of fish under climate-driven heatwaves, supporting the sustainability of marine aquaculture.

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ANTIBACTERIAL ACTIVITY OF *Annona glabra* L. LEAF EXTRACT AGAINST PATHOGENIC BACTERIAS IN AQUATIC ANIMALS

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This study was conducted to evaluate the antibacterial efficacy of *Annona glabra* L. leaf extract against prevalent pathogenic bacteria in aquatic animals. Amidst the complex dynamics of infectious diseases and the escalating threat of antimicrobial resistance, the application of natural botanical compounds has emerged as an imperative strategy for sustainable aquaculture.

The extraction of *A. glabra* leaves was performed using various solvents, among which 96% ethanol yielded the optimal extraction efficiency and antibacterial activity. Antibacterial assays were conducted on *Vibrio fluvialis* strains clinically isolated from fish exhibiting ulcerative syndrome, and subsequently extended to two other critical pathogenic genera: *Aeromonas* and *Streptococcus*.

The results demonstrated that the *A. glabra* leaf extract exhibited highly potent antibacterial properties. Against *V. fluvialis*, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined to be 50 mg/mL and 100 mg/mL, respectively. Notably, complete bacterial eradication was achieved within a mere 5-minute exposure at the MBC. Furthermore, the antimicrobial susceptibility test (antibiogram) revealed that the extract's antibacterial efficacy (exhibiting a zone of inhibition of 20.00 mm) was comparable to conventional antibiotics such as Doxycycline and Cefotaxime, and significantly superior to Erythromycin, Gentamicin, and Ampicillin. Subsequent assays on *Aeromonas* and *Streptococcus* similarly recorded substantial inhibitory and bactericidal effects.

In conclusion, *A. glabra* leaf extract demonstrates significant potential for the development of safe, environmentally friendly bioproducts as viable alternatives to antibiotics for the prophylaxis and treatment of bacterial infections in aquaculture.

GENERAL MANAGEMENT OF SHRIMP DISEASE AT COMMERCIAL SCALE

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Shrimp diseases continue to be one of the major constraints to sustainable aquaculture, causing significant economic losses worldwide despite continuous advances in diagnostics, nutrition, genetics, and health management. While considerable research has focused on individual pathogens and specific intervention strategies, successful disease control at commercial scale requires an integrated management approach that combines prevention, early detection, and continuous optimization of farming practices.

This presentation summarizes ShrimpVet's practical experience in developing and implementing integrated shrimp health management strategies through our Environmental, Social, and Governance (ESG) Demonstration Farm, a 30-ha commercial shrimp farming operation located in the Mekong Delta, Vietnam. Serving as a real-world innovation platform, the ESG Farm enables new technologies, management practices, and health solutions to be validated under commercial production conditions before their wider industry adoption.

Drawing upon extensive field experience, the presentation will discuss the key pillars of commercial shrimp disease management, including biosecurity implementation, water quality control, nutritional optimization, health surveillance, disease diagnostics, microbial management, and rapid response strategies during disease outbreaks. Rather than addressing individual pathogens in isolation, emphasis will be placed on strengthening shrimp robustness through integrated farm management that minimizes production risk and improves system resilience.

Several commercial case studies will demonstrate how data-driven decision-making, continuous farm monitoring, and multidisciplinary management can effectively reduce disease incidence while improving survival, productivity, and production consistency. Practical examples will also illustrate how innovation can be successfully translated from research into scalable commercial farming solutions.

The experiences presented provide a practical framework for shrimp producers, veterinarians, researchers, and aquaculture professionals seeking sustainable and commercially applicable strategies for disease prevention and health management. By bridging scientific knowledge with large-scale farming experience, this presentation highlights how integrated management systems can enhance shrimp health, improve farm performance, and support the long-term sustainability of the global shrimp aquaculture industry.

TASC-RESTOREMED OPEN CALL 1: FROM MISSION AMBITION TO COMMUNITY ACTION IN THE MEDITERRANEAN SEA BASIN

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Introduction

TASC-RestoreMed (Technical assistance and support to communities of actors for the Mission Restore our Ocean and Waters by 2030 for the Mediterranean basin) is a four-year Horizon Europe initiative launched in September 2025 under the European Union Mission “Restore our Ocean and Waters by 2030”. It supports transformative change across the Mediterranean basin by combining financial support to third parties, technical assistance, capacity building, networking, innovation and policy integration. Its approach links ecosystem regeneration with community well-being and supports the Mission’s three pillars: protecting and restoring ecosystems and biodiversity, preventing and eliminating pollution and advancing a sustainable, carbon-neutral and circular blue economy.

Open Call 1 and response

Open Call 1 offered two complementary pathways. Type A - Community-Led Actions supports the deployment, demonstration and scale-up of mature solutions in real operational environments, with grants of €200,000-€1.4 million and a 24-month duration. Type B - Transition Agenda Development supports policy, governance and strategic regional or local roadmaps, with grants of up to €100,000 and a 12-month duration. The call generated a strong Mediterranean response: 225 applications involving 841 applicant entities. Of these, 149 Community-Led Action proposals involved 728 entities, while 76 Transition Agenda Development proposals involved 113 entities.

Results and Mediterranean reach

Community-Led Action proposals were led from 22 countries. Italy accounted for 42 applications (28.19%), Spain 32 (21.48%) and Greece 28 (18.79%). The primary Mission pillar distribution was 60 proposals for ecosystem and biodiversity protection and restoration, 73 for pollution prevention and elimination and 16 for the sustainable blue economy. Small and medium-sized enterprises formed the largest Community-Led Action applicant group (165 entities), followed by non-governmental organisations (142), research/education organisations (128), regional and local authorities (104), associations/foundations (88) and startups (32). Five partners was the most common Community-Led Action consortium size, occurring in 35 proposals.

For Transition Agenda Development, the 76 applications were almost evenly distributed across the three Mission pillars (24, 26 and 26 proposals respectively). The most frequently selected focus areas were environmental issues (54 applications), education/capacity building/social engagement (45), biodiversity (35), governance (34), policy and legal innovation (34) and economic impact (34). Thirty-nine proposals were submitted by a single applicant and 37 by two applicants, illustrating the track’s targeted governance and roadmap-development model.

Relevance for aquaculture

Aquaculture represents a cross-cutting opportunity within this framework. Community-Led Actions can support nature-positive aquaculture, biodiversity monitoring and habitat restoration; water-quality early-warning for shellfish farming, seafood contaminant tracking and nutrient or pollution reduction; and lower-carbon production, circularity, resource efficiency, innovation and skills. Transition Agendas can complement deployment by addressing enabling governance, policy and permitting pathways. The breadth of Open Call 1 therefore demonstrates substantial capacity to connect Mediterranean communities, public authorities, research organisations, civil society and innovative small and medium-sized enterprises around practical and policy solutions relevant to sustainable aquaculture.

Conclusion

The scale and diversity of the response to Open Call 1 indicate strong demand for place-based implementation and governance support aligned with the Mission Ocean objectives. TASC-RestoreMed provides a mechanism for translating Mission ambition into community-led action while creating opportunities for aquaculture to contribute simultaneously to ecosystem restoration, cleaner waters and a resilient circular blue economy.

Acknowledgment

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Reference

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INTEGRATION OF *Hermetia illucens* INTO RAS SYSTEMS: BIOCONVERSION OF AQUACULTURE SLUDGE AND PRODUCTION OF HIGH VALUE-ADDED BIOMASS

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Introduction

Aquaculture systems, particularly recirculating aquaculture systems (RAS), requires a strategy for the sustainable management of the associated sludge (rich in organic matter, nitrogen, and phosphorus). In this context, the black soldier fly (*Hermetia illucens*, BSF) rearing has established itself as one of the most promising biotechnologies offering multiple benefits, including bioconversion capability, waste volume reduction and nutrient upcycling. Despite this conversion efficiency, the use of aquaculture sludge as a growth substrate for insects raises biological and chemical concerns, foremost because of the metals (i.e. copper and zinc) and contaminants bioaccumulation risks along the feed chain mediated by *H. illucens* larvae. For this reason, Regulation (EC) No. 1069/2009 and Regulation (EU) 2017/893 strictly define the substrates permitted for insects rearing, classified as “Farmed Animals”, and currently the use of aquaculture sludge, sewage, or animal feces as a substrate for insects is strictly prohibited. This study explored the potential of the black soldier fly as a bioconversion agent of the sludge derived from the rainbow trout (*Oncorhynchus mykiss*) RAS pilot system of the University of Udine focusing on its potential bioaccumulation in heavy metals, in order to contribute to the development of a sustainable approach to the management of aquaculture by-products, in line with the principles of circular economy.

Materials and methods

BSF larvae were reared using four substrates characterised by increasing levels of sludge (F) in combination with wheat bran (C): F0:C100, F30:C70, F60:C40 and F90:C10, according to a completely randomised experimental design (three replicates per substrate). The larvae were reared under controlled environmental conditions ($T = 40 \pm 1^\circ\text{C}$, $\text{RH} = 75 \pm 3\%$) inside plastic containers ($30 \times 30 \times 20$ cm) until the prepupal stage was reached. In each container, 500 larvae (total weight 2,8 g) younger than 5 days were inoculated in 750 g of substrate. Growth performance were monitored at 2 days basis until the emergence of the first prepupa. The bioconversion efficiency of the substrate was determined by analysing larval growth and initial substrate quantity. The proximate composition and mineral content of the substrates, prepupae, as well as the *frass*, were assessed, along with the potential transfer of heavy metals from the substrates to the larvae.

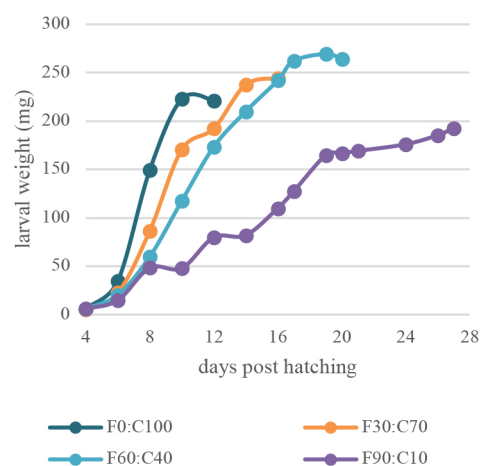


Figure 1 Effect of rearing substrate on the growth of *Hermetia illucens* (average larval weight).

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Results

The co-feeding strategy proved to be effective and the BSF larvae completed their development in all substrates, indicating that aquaculture sludges can be used for the bioconversion process mediated by *Hermetia illucens* (Fig. 1). However, the composition of the substrate significantly affected both the final weight of the prepupae and their development time ($p < 0,05$). The best performance (average prepupal weight) was achieved with the F60:C40 substrate ($269,44 \pm 2,07$ mg), followed by F30:C70 ($244,03 \pm 20,47$ mg). However, increasing the proportion of sludges in the substrate ($>60\%$) led to longer time required for pupation: the F90:C10 substrate resulted in the longest development time (26 days) whereas the F0:C100 substrate enabled the fastest larval development (12 days). Overall, the F30:C70 treatment showed the best bioconversion performance, with a feed conversion ratio (FCR) of $0,92 \pm 0,09$ and a biomass conversion ratio (BCR) of $20,74 \pm 1,35\%$, whilst the worst performance was observed in F90:C10, with an FCR of $2,53 \pm 1,00$ and a BCR of $9,91 \pm 2,59\%$. Substrate composition significantly affected ($p < 0,05$) larvae protein (Kjeldahl N*4,76), lipid, ash and chitin content (ADF–ADL) of the larvae, as well as their fatty acid and mineral composition. The F0:C100 larvae showed the highest protein content ($35,14 \pm 0,32\%$), whilst those reared on F90:C10 resulted in the lowest one ($25,28 \pm 0,68\%$). Conversely, F60:C40 maximised larvae lipid content ($39,39 \pm 0,43\%$), whilst the lowest lipid content was recorded with F30:C70 ($3223 \pm 1,05\%$).

Regarding mineral composition, all substrates registered values within the limits set by European legislation (EC 2002/32), including heavy metals such as Lead (Pb) and Cadmium (Cd).

However, considering the larvae, Copper (Cu) and Zinc (Zn) levels increased together with the amount of sludge in the substrate, exceeding the limits set by Regulation (EC) No 1334/2003 in all substrates excluding F0:C100. The concentration of Manganese (Mn), on the other hand, increased as the proportion of wheat bran rose and exceeded the limits in all substrates excluding F90:C10. Iron (Fe) exceeded the limits only in larvae reared on F90:C10.

The reduction in mineral concentration observed in the *frass* indicates a partial transfer of Copper, Iron, Manganese and, above all, Zinc from the substrate to the larvae.

These processes can represent a risk factor, and highlight the need for monitoring procedures to ensure the safety of possible BSF-mediated bioconversion applications of sludges.

Discussion and conclusion

The results of this study suggest that aquaculture sludge could potentially contribute in the bioconversion processes of BSF, particularly, as part of a balanced co-feeding strategy with wheat bran. The inclusion of up to 60% of fish sludge (F60:C40) in a wheat bran-based diet proved to be effective in terms of BSF bioconversion efficiency and growth performance. At the same time, however, bioaccumulation of selected mineral elements was observed in the BSF larvae confirming the need to monitor the chemical and biological risks associated with the use of aquaculture sludges for insect production. The results open the possibility to investigate the utilization of aquaculture sludge for the production of innovative, high value-added biomass within circular production systems amplifying their direct utilization for bioenergy production systems while support current legislative barriers within the European Union to ensure the production of safe products for the development of alternative production chains.

Acknowledgment

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INCENTIVIZING THE SEPARATION OF FARMED SALMON FROM SEA LICE: A NEW APPROACH TO REGULATING ATLANTIC SALMON FARMING IN NORWAY

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Background

Norway's salmon farming industry is entering a period of fundamental change. A government white paper (Meld. St. 24, 2024–2025) has proposed a new system of lice quotas at the level of each production area, calibrated so that mortality of wild salmon caused by salmon louse stays below 10%. Estimates suggest that, in several production areas, this would require an order-of-magnitude reduction in lice levels at farms compared with current practice (Sandvik et al., 2025). Reaching such targets with today's open-cage technology is not realistic. It may require investments of more than ten billion Euro in shielded production methods such as sea based semi-closed and closed containment systems, and deep or submerged farming – an investment programme of unusually large scale for the sector.

A regulatory framework that drives such a transition must offer companies enough predictability to commit capital, and the regulatory incentive design is critical (Cojocaru, 2024). The underlying biological and environmental processes are highly variable and difficult to measure: lice counts at a single farm fluctuate widely, neighbouring sites infect each other, and the link between counts on farms and mortality of wild salmon must be inferred from incomplete data. The central question is how to reduce lice pressure on wild salmon without imposing risks on companies that are too large for the technology transition to be financed and carried out.

Findings and regulatory designs

Lice quotas, on their own, regulate something that is hard to measure precisely - lice counts on farmed fish - rather than what actually matters for wild salmon - the release of infectious lice larvae and the resulting infection pressure on wild smolts. Counts at a farm are noisy, infection drifts between sites, and the link to lice induced wild-salmon mortality must be modelled with substantial uncertainty. Rather than tightening such a noisy signal, regulators can more effectively reward the physical separation between farmed salmon and lice, which is observable through the choice of production technology and more verifiable through engineering specifications.

We compare different regulatory designs, from lice quotas to incentives for separating farmed salmon and sea lice. A stochastic firm-level optimisation model is developed for a risk averse salmon producer that faces simultaneously a Maximum Allowable Biomass (MAB) cap and a binding lice quota, where the lice population is stochastic.

A firm-level analysis of a salmon producer operating under both a biomass cap and a lice quota, with uncertain lice prevalence and sanctions for breaches, yields several findings. First, even when a company aims at an expected lice level well below the quota, year-to-year variation means that the upper tail will sometimes cross the threshold, generating an expected sanction cost that is invisible in deterministic analyses. Cautious firms therefore stock and grow less than the lice-quota number alone would suggest, providing a firm-level explanation for the substantial production losses the industry would face under tight quotas. Second, a verified low-lice technology reduces both the *average* lice load and its *variability*, whereas a tighter lice quota only moves the threshold without addressing the underlying variability. Technology-based regulation may hence be more attractive than emission-based regulation for any firm averse to downside risk: it lowers expected compliance costs and works as insurance against year-to-year variability the industry cannot control.

Policy implications for Norwegian salmon aquaculture

For Norway, analysis indicate that the most promising package may combine a moderate conversion factor with a regional cap so that converted capacity displaces open-cage exposure within each production area, and the existing farm-level lice limits retained as a backstop for open cages. The analysis suggests that regulating verifiable physical separation between farmed salmon and lice is more effective and less risky for the industry than tightening noisy lice counts – and may be essential for a successful transition of the magnitude that Norwegian salmon farming now faces.

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Acknowledgment

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DIGITAL TECHNOLOGIES FOR SECURE SEAFOOD TRACEABILITY AND COLD-CHAIN MANAGEMENT

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Background and objective

During seafood logistics, even short cold-chain disruptions and limited data visibility can compromise product quality, increase waste, and weaken trust along the value chain. Work Package 6 of the Horizon Europe NOVAFOODIES project addresses these risks by introducing a digital approach that combines continuous cold-chain monitoring with secure, end-to-end traceability across the aquaculture seafood value chain using the following technologies:

- GNSS + IoT, which enables continuous monitoring of location and key environmental parameters during transport, reducing the risk of undetected cold-chain failures.
- Blockchain, which secures critical traceability records through cryptographic mechanisms, ensuring data integrity and resistance to tampering.
- RFID and QR-code technologies, which support fast identification and near-real-time shipment updates, simplifying operations for logistics actors and retailers.

Experimental design

We developed a digital traceability ecosystem that combines:

- An IoT-based system and device for real-time information, providing data on environmental conditions and indicators related to product quality and degradation during logistics.
- An online Marketplace for seafood products, supporting producers in managing batches, shipments, and traceability data, including quality and biosecurity information. The platform targets at least 100 operators.
- A mobile app for consumers and end-users, enabling users to access verified product information by scanning QR codes at the point of sale, increasing transparency and trust. The pilot targets at least 1,000 users.

We applied this ecosystem in two pilot cases: Aqua De Mã in Italy and Kefalonia Fisheries in Greece, demonstrating that the architecture can be applied across different seafood supply-chain and logistical settings.

Results and discussion

Table 1 summarizes the main differences between traditional seafood traceability and cold-chain management practices and the NOVAFOODIES approach, together with the expected operational, quality, and transparency benefits.

Conclusions

Integrating digital tools into a unified ecosystem enables more effective cold-chain control and end-to-end seafood traceability. Continuous monitoring and secure digital traceability replace fragmented records, supporting product quality, risk management, and consumer trust. The approach is scalable and transferable, enhancing sustainability and transparency across European value chains.

Acknowledgments

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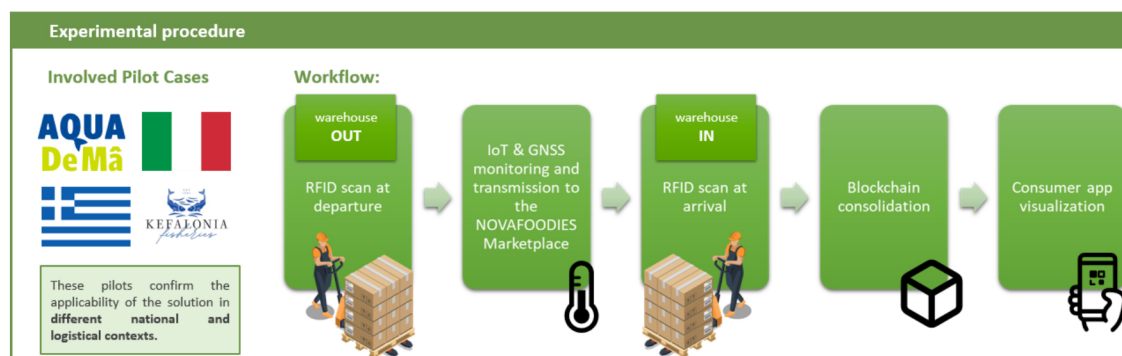


Figure 1: Experimental design

Table 1 : Comparison of traditional seafood traceability and cold-chain management practices with the NOVAFOODIES digital approach and its expected impacts.

Traditional approach	NOVAFOODIES approach	Impact
Spot checks at handover points; blind time between checkpoints.	Continuous, time-stamped IoT monitoring linked to batch and shipment ID.	Earlier temperature-excursion detection, better decisions, and lower spoilage risk.
Each actor stores data separately.	Single digital record per batch or shipment in the Marketplace, enriched by IoT data.	Less rework, fewer mismatches, and smoother handovers across the chain.
Records can be edited or lost across organisations.	Blockchain anchoring makes key records tamper-evident.	Clearer accountability.
Manual paperwork; limited real-time location information.	RFID and QR-code scans, together with GNSS monitoring, create event-based shipment tracking.	Faster receiving, fewer errors, and quicker issue isolation.
Static label information; consumers rely mainly on brand claims.	A QR-code scan shows the batch story in the app, with data authenticity supported by blockchain.	Higher perceived trust and clearer communication of product quality and origin.
Deviations are detected late.	Automated alerts for abnormal conditions enable faster escalation and response.	Reduced waste, improved shelf life, and fewer rejected deliveries.

DIGITAL PHENOTYPING IN FISH TELEMETRY

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Digital phenotyping in aquaculture

Digital phenotyping is increasingly used in aquaculture to extract biologically and operationally relevant information from large volumes of sensors data. To date, most applications of digital phenotyping in farmed fish are based on image and video monitoring systems combined with artificial intelligence. Such approaches were successfully applied on feeding behavior, swimming patterns, biomass estimation and external welfare indicators. However, despite the advances, image based systems are also inherently limited to externally observable behavior and are sensitive to visibility, illumination and camera positioning. Behavior occurring at depth, during night time, or under poor visual conditions remains largely inaccessible. In addition, internal physiological responses to environmental or operational challenges are difficult to analyze using visual data alone.

Telemetry as under-used source for digital phenotyping

At the same time, fish telemetry and biologging technologies are already well established. Acoustic telemetry of various sensors is widely used to study behavior, energetics and stress responses. They are typically analyzed using isolated metrics or descriptive summaries and only rarely framed within explicit digital phenotyping paradigm. The potential of telemetry to provide integrated, interpretable and operational phenotypes remains under utilized.

Here, digital phenotyping provides a unifying conceptual framework for telemetry based aquaculture monitoring by explicitly linking raw sensor data to biologically meaningful response profiles. Instead of treating telemetry outputs as standalone metrics, digital phenotyping emphasizes systematic data quality control, feature aggregation across temporal scales, and the synthesis of multivariate patterns into individual level digital phenotypes. These phenotypes can represent functional traits such as activity regulation, stress sensitivity, resilience, or recovery dynamics, bridging telemetry, physiology, and welfare together. Digital phenotyping does not replace existing telemetry methodologies, but reframes them as a phenotype centric pipeline with direct relevance for welfare monitoring, early warning systems, and adaptive aquaculture management.

Applications for welfare and management monitoring

Key application is continuous welfare monitoring in aquaculture systems. Digital welfare phenotypes quantify deviations from baseline behavior and physiology following routine husbandry procedures such as crowding, delousing or transfer. By focusing on recovery trajectories rather than single stress indicators, this approach enables objective comparison of welfare impacts across procedures, seasons and production systems.

It may also support early warning and anomaly detection. Gradual shifts in activity patterns, ventilation dynamics or depth use may precede visible signs of welfare compromise or production loss. Monitoring deviations from established phenotypic envelopes provides a basis for identifying emerging risks earlier than traditional threshold or mortality based indicators.

It is not proposed as replacement of existing image based systems. Both approaches are actually complementary. Image based phenotyping provides valuable information on external behavior at specific locations and times, while telemetry based phenotyping offers continuous insight into internal state and behavioral dynamics independent of visibility or daylight. Combined, these approaches have the potential to provide more complete representation of fish phenotypes in aquaculture. Future work should focus on validation across species and production systems, integration with feeding and bioenergetic models, and alignment with farm level decision processes.

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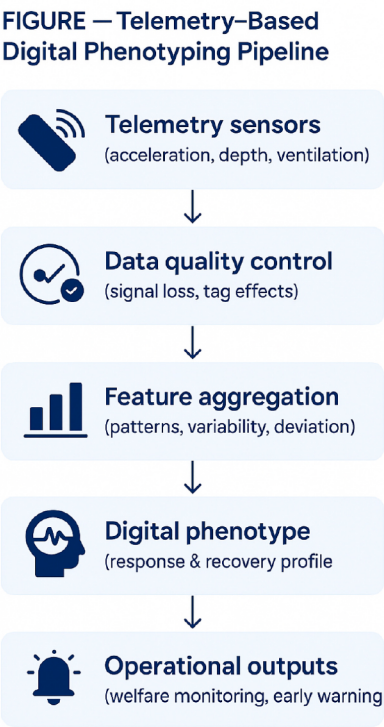


Figure 1. Conceptual diagram of telemetry based digital phenotyping in aquaculture, integrating multi modal sensor signals to characterise internal behavioural and physiological dynamics as continuous phenotypic trajectories.

FROM POND TO PLATE: ANIMAL WELFARE CHALLENGES AND FUTURE DIRECTIONS IN COMMON CARP (*Cyprinus carpio*) AQUACULTURE

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Introduction

Common carp (*Cyprinus carpio*) is a key species in European freshwater aquaculture, with a production history exceeding 900 years and a strong socio-economic and cultural relevance, particularly in Central and Eastern Europe (Urbányi, 2026). In addition to its importance in sustainable, low-input pond farming systems, carp production is increasingly influenced by societal expectations and regulatory developments related to animal welfare. Despite this, welfare assessment and management in carp farming remain less standardized compared to terrestrial livestock systems.

Materials and Methods

Animal welfare challenges occur throughout the entire production cycle, but are particularly pronounced during handling, harvesting, transport, and slaughter. Existing regulatory frameworks, such as Council Regulation (EC) No 1/2005, acknowledge welfare risks during transport; however, they were primarily developed for terrestrial animals and are only partially adapted to fish (Jung-Schroers, 2026). During routine management procedures, carp are exposed to multiple stressors, including crowding, netting, sorting, and temporary air exposure. These can result in physiological stress, injuries to skin, fins and gills, and increased susceptibility to disease.

Results

Live transport represents a critical control point for welfare. Key risk factors include high stocking densities (typically 330–500 kg m⁻³), fluctuations in water quality (oxygen, temperature, pH), and accumulation of metabolic by-products such as ammonia and carbon dioxide (Kuczyński, 2026). Inadequate oxygen supply (<4 mg L⁻¹), rapid temperature changes, and prolonged transport duration are associated with increased mortality and sublethal stress effects. Legal and practical considerations emphasize that fish must be transported in an aquatic environment that ensures species-specific needs, with improper practices potentially constituting animal welfare violations (Kuczyński, 2026).

Operational Welfare Indicators (OWIs) have been proposed as practical tools to assess welfare in carp under farm conditions. These include environmental parameters (e.g. oxygen saturation, pH), group-level indicators (mortality rates), and individual-based indicators (injuries, behavioural changes). Among these, oxygen levels, pH, mortality, and injury rates are currently considered the most reliable and feasible indicators for routine application (EFSA, 2008). However, the high environmental tolerance of carp complicates the interpretation of these indicators and may mask suboptimal welfare conditions.

Discussion

Future research and development should focus on the refinement and validation of species-specific welfare indicators, including non-invasive and real-time monitoring approaches. Technological innovations in transport systems (e.g. improved tank design, continuous water quality monitoring) and standardized handling protocols are essential to reduce stress and mortality. Furthermore, adaptation of existing legislation to fish-specific requirements and the development of welfare certification schemes could support improved practices and market acceptance (Heistinger, 2026). Strengthening interdisciplinary collaboration between aquaculture, veterinary science, and animal behaviour research will be crucial to advance welfare-oriented carp production.

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Acknowledgment

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WATERDUNEN: INTEGRATING AQUACULTURE, TIDAL NATURE AND PUBLIC ENGAGEMENT IN A MULTI-USE COASTAL LANDSCAPE

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The Waterdunen area

Waterdunen, located near Groede, in the southwestern part of the Netherlands, is a man-made saline tidal landscape developed following the reinforcement of the primary coastal defence system. The 300 ha area combines estuarine nature development, recreation and aquaculture within a 3.2 km tidal creek system. North Sea water enters the system through culverts that dampen tidal fluctuations, resulting in a tidal range of approximately 1.2 m. Around 30 ha of the site is designated for aquaculture activities. Opened to the public in 2022, Waterdunen is still under development and is expected to attract approximately 100,000 visitors annually.

Aquaculture production

Since 2020, experimental aquaculture activities have focused on the cultivation of Pacific oysters (*Crassostrea gigas*) and saline crops, including glasswort (*Salicornia europaea*) and sea aster (*Tripolium pannonicum*). The saline crops are cultivated along the creek shorelines, while oyster production takes place within a designated 7,500 m² area at the end of the creek system. Oyster growth rates have exceeded expectations, although the currently allocated production area appears insufficient for fully commercial-scale production. Nevertheless, the site has proven highly suitable as a demonstration and education location for aquaculture. Public accessibility through an extensive network of footpaths, combined with the limited tidal range, provides visitors with clear visibility of both oyster cultivation systems and saline crop production sites.

Public engagement

Due to its unique integration of tidal nature, recreation and aquaculture, Waterdunen offers significant opportunities to strengthen public awareness and social acceptance of aquaculture. Several outreach and educational activities have therefore been implemented, including informational panels at production sites, training sessions for local nature guides, and regular guided excursions. During 2024, approximately 60 guided tours were organised in the area. These activities contribute to increase public understanding of sustainable aquaculture practices and demonstrate the potential of multifunctional coastal landscapes to support social licensing of aquaculture.

UNLOCKING THE WIDER POTENTIAL OF AQUACULTURE IN CIRCULAR FOOD SYSTEMS IN EUROPE

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Introduction

Aquaculture is a vital food source around the globe, playing an important role in future food systems, by providing nutrients to the human diet. Yet, its potential to upcycle biomass and contribute to nutrient supply in circular food systems remains largely unstudied. Circular food systems are seen as a promising way forward to reduce the environmental impacts of food systems. A key principle of circular food systems is using biomass effectively by prioritising it as food for humans. Farm animals, including aquaculture species, should consume resources that are inedible for humans, such as grass, food waste and by-products from crops, livestock and fisheries. The availability of these so called low-opportunity-cost feeds (LCF) may constrain aquaculture growth. Aquaculture species vary in their ability to upcycle LCF into food, and the implications for nutrient supply in food systems are unclear. In this study, we provide insight into 1) what nutrients aquaculture can supply to the human diet, 2) how much fish can be produced when animals are fed exclusively with LCF, and 3) which LCF can be recycled into fish feed. Answering these questions allows the quantification of how much aquaculture is needed in circular food systems, and therefore, the discussion of the space required for aquaculture.

Methods

We extended the resource-allocation models of van Hal et al. (2019) and van Selm et al. (2022) to develop FEEDSOM (FEED Systems Optimization Model). FEEDSOM is a linear programming optimization model that allocates LCF across livestock and aquaculture systems to meet human nutrient requirements in Europe (EU-27 plus the United Kingdom, Iceland, and Norway). Livestock and aquaculture species were fed only on LCF. Capture fisheries are represented by the average landings of the twenty most caught species in Europe (2000-2020). Aquaculture is represented by Atlantic salmon and European seabass (carnivorous species) and common carp (omnivorous freshwater species). The model provides insights in how animal-sourced food can complement plant-sourced foods to supply nutrients, including protein, vitamin B12, calcium, and the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The objective of FEEDSOM was to maximise the number of people whose nutritional requirements can be met. We compared a baseline scenario, where aquaculture and fisheries are limited to current production and consumption (Reference), with two alternative scenarios: 1) FarmMore: expanding aquaculture 2) EatMore: all food grade fish is consumed and edible yields are extended.

Results and Discussion

Meeting the nutrient requirements while animals are fed exclusively with LCF resulted in 18 grams of fish per capita per day in the EatMore scenario, which is lower than the EAT-Lancet recommendations (28 g/d) (Willett et al., 2019). Expanding aquaculture increased fish consumption to 31 g/d, but at the cost of reduced livestock production. The carnivorous species, Atlantic salmon and European seabass, were fed high quality animal by-products, such as fishmeal, blood meal and by-product meal. Carp were fed low-quality animal by-products, such as meat and bone meal, and high-quality plant products, such as oil crop meal.

In this study, EPA/DHA were the limiting nutrients and the main driver of aquaculture production. Carnivorous aquaculture species can play an important role in circular food systems by upcycling EPA/DHA from fisheries by-products. However, the dependence of carnivorous aquaculture species on these limited resources limits their capacity to expand production. In contrast, omnivorous aquaculture species do not rely on fisheries inputs to supply EPA/DHA, but their overall contribution to EPA/DHA supply is relatively low.

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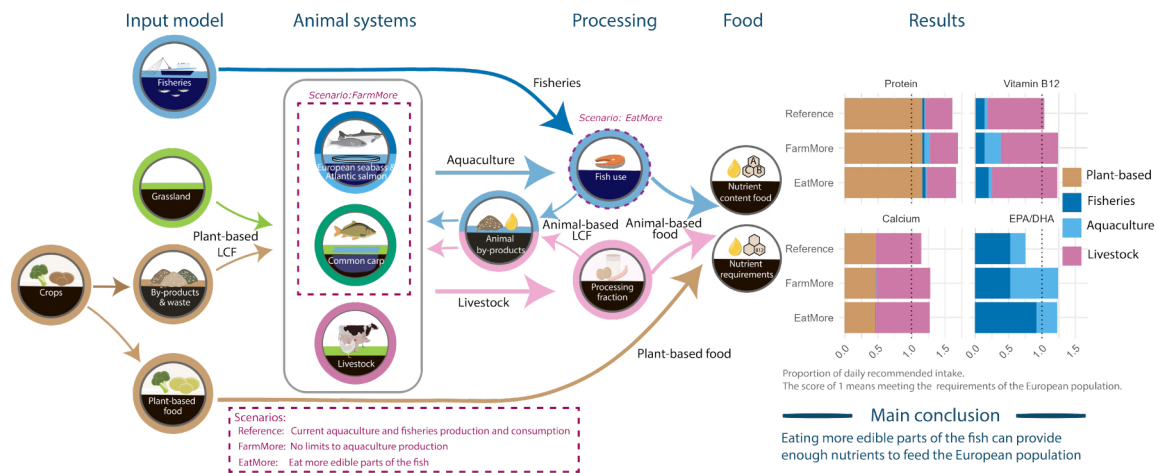


Figure 1 Contribution of aquaculture and fisheries to nutrient supply in circular food systems in Europe in the Reference, EatMore, and FarmMore scenarios using the Feed Optimisation Model (FEEDSOM). Arrows indicate biomass flows. LCF: low-opportunity-cost feed; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid

Under current aquaculture production and consumption, we cannot supply enough EPA/DHA to meet the European nutrient requirements. To reduce this nutrient gap, we could increase aquaculture production (Figure 1, FarmMore scenario). However, expanding aquaculture should not be the priority, as it requires more LCF, production area, and increases environmental pressure. A more efficient strategy is to eat more edible parts of the fish (Figure 1, EatMore scenario). This approach could meet European nutrient requirement, including EPA/DHA, while also producing enough animal-sourced nutrients to help feed an additional 118 million people outside Europe. **Figure 1 Contribution of aquaculture and fisheries to nutrient supply in circular food systems in Europe in the Reference, EatMore, and FarmMore scenarios using the Feed Optimisation Model (FEEDSOM).** Arrows indicate biomass flows. LCF: low-opportunity-cost feed; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid

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LOW MAINTANANCE HIGH IMPACT SEAWEED PRODUCTION IN MULTI-USE PLATFORMS

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Introduction

Intensive finfish and shellfish aquaculture can impose significant pressures on surrounding ecosystems through the release of dissolved nitrogen and phosphate, potentially leading to eutrophication and its associated ecological impacts. One promising strategy to mitigate these impacts is the implementation of an integrated multitrophic aquaculture (IMTA) system, whereby extractive organisms at lower trophic levels assimilate dissolved nutrients and organic matter generated by fed, higher-trophic species.

In parallel, marine aquaculture development is increasingly constrained by the limited availability of suitable sites and growing competition for marine space from other sectors, including fisheries, shipping, renewable energy, and conservation initiatives. One potential approach to addressing this challenge is the development of multi-use platforms, where the co-location of complementary marine activities enables more efficient use of space while providing environmental and economic benefits.

In this context, we present a demonstration study in which a seaweed cultivation system was deployed downstream of a salmon farm to evaluate nutrient recovery and ecological enhancement. In addition, a case study is presented that explores the integration of seaweed cultivation within an offshore wind farm as an example of a multi-use marine platform.

Demonstration

Seaweeds are low-trophic photosynthetic organisms with a high capacity for assimilating dissolved inorganic compounds such as ammonia, nitrate and inorganic phosphate. Krupandan *et al.* (2026) reported a strong connection between the salmon aquaculture and adjacent seaweed cultivations with 92.8% of the nitrogen assimilated by the seaweed originating from salmon derived nutrients/waste inputs based stable isotope analysis.

The present study built on these findings by investigating the influence of salmon aquaculture on seaweed growth while simultaneously assessing the role of seaweed cultivation in supporting native biodiversity. To achieve this a seaweed farm was deployed downstream of a commercial salmon farm in Bantry Bay, southwest Ireland, to capture nutrients released during salmon production. A control site was established in Toormore Bay to provide a reference for seaweed growth and biodiversity.

Direct comparisons of seaweed growth between the two locations should be interpreted with caution, as environmental differences between the sites are expected to influence growth performance independently of aquaculture-derived nutrient inputs. Consequently, the control site primarily serves as a baseline for biodiversity assessments.

Results from 2025 indicated greater seaweed growth at the control site than at the IMTA site (Table 1). Seawater nutrient analyses suggest that this difference was likely associated with elevated nutrient concentrations at the control site during that year. In contrast, this trend was not observed in 2026. Ongoing analyses of seawater samples will determine whether this observation can be attributed to interannual variation in nutrient availability or other environmental factors.

A further finding shows a substantially greater abundance of epifauna at the IMTA site relative to the control site. Significant differences between the two study years at the IMTA location were observed when the salmon cages were stocked and fallow. Further statistical analyses will investigate whether these patterns are associated with the presence of salmon farming activities and the resulting environmental conditions.

Table 1 Seaweed harvest yield and epifauna abundance per seaweed sample.

	Bantry Bay, IMTA		Toormore Bay, Control	
	2025	2026	2025	2026
Average wet weight (kg/m)	11.66 ± 3.04	13.57 ± 6.48	17.35 ± 6.71	11.57 ± 4.63
Epifauna abundancy	507	1211	158	128

(Continued on next page)

We hypothesise that seaweed cultivation create habitats that support higher abundances of epifaunal organisms. In turn, these communities may attract larger mobile fauna and contribute to increased local biodiversity and ecosystem functioning. Further analyses will determine the extent to which seaweed farms act as biodiversity enhancement structures and whether these effects are influenced by proximity to salmon aquaculture operations.

Multi-Use Platform Case study

Commercial kelp cultivation typically requires relatively low levels of maintenance following deployment and does not require external nutrient inputs. Routine management is generally limited to periodic inspections and maintenance following severe weather events. These characteristics make kelp cultivation a promising candidate for co-location with offshore wind energy infrastructure.

In this case study, we propose the integration of kelp cultivation within an offshore wind farm. While areas immediately surrounding turbine foundations and navigation corridors must remain accessible, a substantial proportion of the space between turbines remains largely unused. This available area could support seaweed cultivation, thereby increasing the productivity and economic value of existing marine infrastructure.

Co-location may also offer operational efficiencies through the sharing of vessels, personnel, and maintenance resources between wind farm and seaweed farm operators. Furthermore, cross-training of wind farm personnel could facilitate routine seaweed farm inspections and maintenance, reducing operational costs.

The adoption of innovative cultivation technologies, such as depth-adjustable longline systems, could further improve the feasibility of offshore seaweed production. These systems remain at the sea surface under favourable conditions to maximise light availability and growth, but can be submerged during periods of rough weather to reduce mechanical stress and minimise the risk of infrastructure damage. Such approaches could enhance the resilience and economic viability of seaweed cultivation within offshore renewable energy developments.

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LOCAL ADAPTATION OF EUROPEAN SEABASS (*Dicentrarchus labrax*) POPULATIONS TO TEMPERATURE AS REVEALED BY SEASONAL GROWTH RATE AND GENETIC PARAMETERS IN THREE TEMPERATURE REGIMES

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Introduction

With global warming, understanding the temperature effect on fish growth is essential for sustainable aquaculture. The European seabass is an ideal model for this, as a key aquaculture species in the Mediterranean, which is strongly affected by global warming. We tested the hypothesis that the Atlantic (AT), West Mediterranean (WM) and East Mediterranean (EM) wild populations of European seabass would each perform better in their own temperature regime.

Material and Methods

We evaluated body weight and daily growth coefficient (DGC) of the three populations, as well as their genetic parameters, when reared in common garden, in three thermal regimes that reflected their three native habitats: a cold Atlantic (rAT), an intermediate Western Mediterranean (rWM) and a warmer Eastern Mediterranean (rEM) seasonal temperature regimes. Body weight was measured every six weeks for 2-3 years. All fish were genotyped on the DlabChip 57K SNP chip, allowing *a posteriori* parentage assignment as well as establishing the genomic relationship matrix.

Results and discussion

The AT fish were heavier than WM and EM in all regimes and at all times. The DGC of the AT population was higher than that of WM and EM during the first year, while in the second and third year this was only true for rearing temperatures below 18 °C. Thus, they grew faster at all times in their own rAT regime, and during autumn and winter in rWM and rEM regimes. The EM population had higher DGC in spring and summer in the Mediterranean regimes. Overall, these phenotypic results can be interpreted as local adaptation of the populations to their natural thermal regime. Both temperature and age were associated with declines in the heritability of DGC, but the expected response to selection remained higher in summer, due to higher genetic and phenotypic variance. The genetic correlations (r_G) of DGC between two seasons decreased when the temperature difference between both seasons increased. The EM population had overall higher r_G across seasons than WM and AT, thus showing a potential for more stable growth performance. A residual seasonal effect on r_G showed there are specific seasonal metabolic pathways. The EM population should be preferred for high temperature culture, and based on previous studies, we hypothesize that a terminal cross of AT and EM fish could have a good aquaculture potential.

Acknowledgements

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ADVANCE YOUR AQUACULTURE RESEARCH THROUGH FUNDED ACCESS TO EUROPEAN RESEARCH INFRASTRUCTURES WITH AQUASERV

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LIGHT AS A KEY ENVIRONMENTAL DRIVER OF FISH WELFARE: A REVIEW OF CURRENT KNOWLEDGE

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Light is a fundamental environmental factor shaping the physiology, behavior, and overall welfare of fish. Beyond its role in vision, light acts as a primary regulator of biological rhythms and endocrine processes, influencing a wide range of functions including growth, reproduction, immune activity, and stress responses. This review synthesizes current knowledge on the relationship between light conditions and fish welfare, with a particular focus on photoperiod, light intensity, and spectral composition.

The photoperiod is one of the most predictable environmental cues in aquatic systems and plays a central role in synchronizing circadian and seasonal rhythms in fish (Sánchez-Vázquez 2019). Light signals are transduced via the retina and pineal organ, leading to rhythmic melatonin secretion, which typically peaks during darkness and conveys temporal information to the organism (Bayarri et al 2004). Melatonin acts as a key endocrine mediator, regulating physiological processes such as reproduction, metabolism, and immune function (Maitra and Hasan 2016). In addition, several other hormones and metabolites (e.g. cortisol, GnRH, LH, lactate, glucose, etc.) are affected by photoperiod (Rodriguez et al 2001). Disruption of natural light–dark cycles, for example through continuous illumination, can therefore alter hormonal balance and impair welfare (Liu et al 2026).

In aquaculture, manipulation of photoperiod is widely used to optimize production. Delay of sexual maturation can enhance of growth, in the other hand lightning regime is used for synchronize sexual maturation (Bayarri et al 2004). However, these production-oriented practices may conflict with welfare considerations, as prolonged or unnatural light conditions can disrupt circadian organization and increase chronic stress.

Light intensity and spectral composition further modulate fish responses. Excessive light intensity can induce avoidance behavior and physiological stress, while insufficient light may impair feeding efficiency, particularly in visually oriented species (Li et al 2025). Emerging research indicates that different wavelengths of light can differentially affect physiological processes; for instance, specific light spectra may influence oxidative stress and immune responses in fish, potentially via melatonin-mediated pathways (Jung et al 2016). These findings highlight the importance of considering not only the duration but also the quality of light.

Behavioral responses to light provide additional insight into welfare status. Fish often exhibit clear preferences for specific light environments, such as shaded or low-intensity zones, and changes in light conditions can alter activity patterns, spatial distribution, and social behavior. Such behavioral indicators are increasingly recognized as valuable tools for assessing welfare in both experimental and applied settings (Marchesan et al. 2004).

Despite significant advances, important knowledge gaps remain. Optimal lighting conditions are highly species-specific and depend on life stage, ecological background, and interactions with other environmental factors such as temperature and stocking density. Furthermore, long-term welfare consequences of artificial lighting regimes are still poorly understood. While continuous light may enhance short-term production, it may also lead to maladaptive physiological states and reduced resilience under more natural conditions.

In conclusion, light is a key but complex driver of fish welfare, acting through integrated neuroendocrine and behavioral pathways. Future research should aim to develop species-specific, welfare-oriented lighting strategies that balance production goals with the biological needs of fish, incorporating a more holistic understanding of environmental interactions.

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Hermetia illucens AND *Tenebrio molitor* REARED ON OLIVE POMACE AS FUNCTIONAL FISH MEAL ALTERNATIVE IN *Dicentrarchus labrax* DIET

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Introduction

Alongside exploring alternative sustainable protein sources, aquaculture research intensively investigates ingredients with immunostimulant, growth-promoting, or antimicrobial properties. Insect meals have shown bioactive benefits, including prebiotic, anti-inflammatory, and antimicrobial effects (Biagini et al., 2022). Olive pomace, a byproduct rich in bioactive compounds, is not yet commonly revalorized (Madureira et al., 2022). While insect meal is a promising protein source in aquaculture and some species can bioaccumulate these compounds from feed, using insects as carriers for bioactive substances remains unexplored. This study assessed the impact of feeding European seabass (*Dicentrarchus labrax*) with diets containing *Tenebrio molitor* (TM) and *Hermetia illucens* (HI) reared on olive pomace. We examined growth performance and gut microbiome composition through metagenomic analysis.

Materials and methods

Five isoproteic and isolipidic diets were prepared: control (C), HI (HI-C), HI fed olive pomace (HI-P), TM (TM-C), and TM fed olive pomace (TM-P). After a 14-day acclimatization period, 300 juveniles of *D. labrax* (7.25 g) provided by Predomar S.L. (Carboneras, Spain) were weighed and randomly distributed among five treatments in three tanks (300 L) per treatment. After 48 days, the fish were anesthetized, weighed, and sacrificed. For metagenomic analysis, a pool of nine intestines (3 from each tank) was preserved in a 20% sterile glycerol solution at -80 °C until mass sequencing by SECUGEN S.L. (Madrid, Spain) with Oxford Nanopore technology on a MinION FLO-MIN114 R10.4.1. (Oxford, UK). DNA extraction was performed using the QiAmp PowerFecal Pro DNA kit (Qiagen, Hilden, Germany) according to the protocol described by Lyons et al. (2017). The yield and integrity of the extracted DNA were measured using a NanoDrop 3300 spectrophotometer (Thermo Fisher Scientific, Waltham, USA).

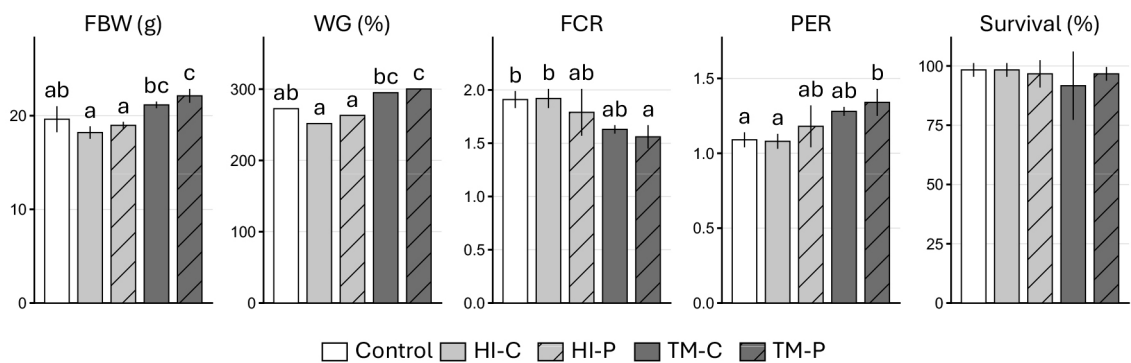


Figure 1. Growth performance and feed utilization. FBW, final body weight; WG, weight gain; FCR, feed conversion ratio; PER, protein efficiency ratio. Means ± SD (n = 3); different letters indicate significant differences ($p < 0.05$).

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Results

The inclusion of HI and TM meals led to changes in the growth parameters of *D. labrax*, as shown in Figure 1. The TM-P diet resulted in significantly ($p < 0.05$) better growth rate (GR) and feed efficiency (FCR) than the control and HI-based diets. The TM-C treatment showed intermediate performance between the C diet and the HI-C and HI-P diets, with no significant differences compared with TM-P. Protein efficiency (PER) was significantly higher ($p < 0.05$) than the control, HI-P, TM-C, and TM-P treatments. Survival rates ranged from 91.67% to 98.33%, with no significant differences among the diet groups. Regarding gut ecology, the microbiota of *D. labrax* fed HI-based diets showed higher diversity than the control and TM-based diets, as indicated by the Chao1 analysis. Additionally, both experimental diets showed greater diversity than the control diet according to the Shannon analysis.

Discussion

Olive pomace, an agricultural byproduct, was effectively utilized as a substrate to rear both *T. molitor* and *H. illucens*, producing meals suitable for inclusion in *D. labrax* diets. Overall, TM meal served as a more efficient fish meal substitute than HI meal: fish fed TM-P achieved the highest final body weight and weight gain, along with the lowest FCR, significantly surpassing the control. In contrast, HI meal did not differ from the control in these measures. However, HI meals increased gut microbial alpha diversity. Thus, the two insect species influenced different aspects of fish performance rather than one being always superior. These findings support the potential of using insects as bioreactors to transform low-value organic side streams into protein-rich aquafeed ingredients.

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FEED-STABILITY OF A FREEZE-DRIED BACTERIAL FORMULATION WITH POTENTIAL PROBIOTIC EFFECTS ON THE REPRODUCTIVE PERFORMANCE OF COMMON CARP (*Cyprinus carpio*)

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Introduction

The rapidly increasing global demand for aquaculture products brings a range of complex challenges that must be addressed to ensure sustainable and efficient production (Rul., at al 2024). The most critical issues are the prevention and control of infectious diseases, broodstock management strategies to maintain high reproductive performance, and the optimization of feeding practices. Probiotics can be the ideal alternative to antibiotics and chemotherapeutics by enhancing immunity, reproduction and digestion (Rahayu et al., 2024). Furthermore, the efficacy of probiotics depends not only on the selection of an appropriate microbial strain but also on the delivery method, dosage, and the ability of the microorganisms to survive feed processing, storage, and passage through the gastrointestinal tract. Feed coating, microencapsulation, and other protective delivery technologies have therefore attracted increasing attention as strategies to preserve probiotic viability and ensure successful colonization of the host intestine (Calcagnile., et al 2024).

Materials and Methods

During the experiment, the surface stability of a newly isolated and described, *Lactiplantibacillus plantarum* (TÉT43) probiotic bacterial strain was investigated. 1.5 g of freeze-dried *L. plantarum* was suspended in 15 mL of PBS (phosphate-buffered saline) and was sprayed onto the surface of 150 g of Aller Aqua Master 6 mm (Allervej130, 6070 Christiansfeld, Denmark) complete feed using a syringe bottle. The treated feed was divided into 50-gram portions, of which 50 grams were treated with 3 mL of sunflower oil and 50 grams with a gelatine coating (0.5 g of edible gelatine dissolved in 10 mL of distilled water). The remaining 50 g of feed was not treated with any additional coating; this served as the control. After drying at room temperature for one hour, 10 g of feed from each of the three groups was placed into conical flasks containing 90 mL of PBS in three parallel runs, then were shaken at 120 RPM at room temperature for 1 hour. After shaking, we decanted the PBS solution, placed the feed into 50-mL Falcon tubes, and vortexed each tube with 10 mL of PBS solution for 90 seconds. We prepared a tenfold dilution series from the tubes, then spread 100 microliters of each dilution onto M-17 medium and incubated at 35 °C for 96 hours. After incubation period, we counted the number of colony-forming units (CFU mL⁻¹) and evaluated the results.

Results and Discussion

The gelatin-coated feed exhibited a significantly higher cultivable bacterial count ($P=0.0219$) than the control feeds, indicating that this treatment provided the greatest surface stability of the tested probiotic on the feed. The applied coating increased the cost by approximately €0.15 per kilogram of feed, representing an economically acceptable increase considering the improved stability and efficacy of the probiotic formulation. The validated feed supplementing method may also reduce dissolution losses and it can improve the utilization of other feed additives or vitamins applied after feed production in the future.

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TEMPORAL PROTEOMIC DYNAMICS ACROSS A 24-HOUR CYCLE IN DIGESTIVE AND SALIVARY GLANDS OF JUVENILE AND SUBADULT *Octopus vulgaris*

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Introduction

Biological rhythms intrinsically regulate a wide range of physiological and metabolic processes, including daily patterns of feeding, digestion, and energy utilization. In marine organisms, particularly cephalopods, the molecular mechanisms underlying these rhythms and their functional significance remain poorly understood. In the common octopus (*Octopus vulgaris*), a species of major importance for fisheries and aquaculture, the posterior salivary glands (PSG) and digestive gland (DG) exhibit distinct but complementary functional specializations that vary across ontogeny. In early life stages, the PSG play a central role in prey immobilization and predigestion through the secretion of toxins, digestive enzymes, and other bioactive compounds, whereas the DG progressively acquires a dominant role in digestion, nutrient absorption, assimilation, storage, and metabolism as it matures. Together, these organs ensure efficient prey processing and nutrient utilization throughout development. Understanding how molecular rhythms regulating digestive function change during the ontogeny of *O. vulgaris* is essential for the development of optimized feeding strategies that support growth performance, improve physiological efficiency, and promote animal welfare under aquaculture conditions. Therefore, this study investigated the protein expression patterns of the posterior salivary glands (PSG) and digestive gland (DG) throughout a 24-hour cycle in recently settled juveniles and subadult *O. vulgaris* using proteomic analyses.

Materials and Methods

Collection and maintenance of specimens: Juvenile and subadult *O. vulgaris* specimens were collected off Vilanova i la Geltrú (northwestern Mediterranean) using experimental collectors deployed at depths of 5–15 m on sandy bottoms (Escolar et al., 2021) and traditional traps set at depths of 5–27 m. Subadults were collected on October 9, 2023, between 05:30 and 10:30 h, whereas juveniles were collected on November 11, 2023, between 06:30 and 11:00 h. Following capture, the animals were kept in containers on board and transported to the ICM-CSIC Experimental Aquarium Facilities, located 50 km away, where they arrived before 13:00 h. At the facilities, specimens were maintained in tanks supplied with a flow-through seawater system under controlled conditions reflecting the natural photoperiod and temperature: 23.9°C for subadults and 17.2°C for juveniles. Light intensity at the bottom of the tanks was 80–90 lux for subadults and 15 lux for juveniles. Local sunrise and sunset times during the experiments were 07:57 and 19:19 h for subadults, and 07:45 and 17:45 h for juveniles, respectively. Specimens were not fed during either the acclimation period or sampling.

Sample preparation for mass spectrometry analysis: Protein extracts were obtained using an SDS-based lysis buffer, quantified by BCA assay, and processed with MagResin Hydroxyl magnetic beads. Samples containing 50 µg of protein were digested overnight with trypsin/Lys-C. Subsequently, 1.5 µg of peptides was desalted using Evotips, dried, and stored at –80°C until MS analysis.

nanoLC-MS/MS analysis: Peptides were analyzed using a timsTOF Pro mass spectrometer coupled to a nanoElute nanoLC system. Chromatographic separation was performed on a C18 column using a 30-min gradient, followed by nanoelectrospray ionization and diaPASEF data acquisition (Arenas-De Larriva et al 2022).

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Raw Data Processing and Protein Identification: Raw diaPASEF data were processed using DIA-NN v2.0 Academia in library-free mode against the *Octopus vulgaris* UniProt reference proteome (Demichev et al., 2020). Trypsin/P specificity, standard fixed and variable modifications, and a precursor-level FDR of 1% were applied. Next, the ProteinGroup.txt file was loaded onto Perseus (v1.6.2.3) (Tyanova et al., 2016) for further processing and statistical analysis. Functional analysis of the proteins identified was done with Iulius AI (<https://julius.ai/>).

Results and discussion

The proteomic analysis of the digestive gland (DG) in subadult specimens revealed that digestion-related enzymes are differentially expressed exclusively in this tissue. In general, these enzymes exhibit higher activity between 08:00 and 16:00, whereas downregulation is mainly observed between 20:00 and 04:00, reaching its lowest level at 00:00. Regarding the posterior salivary gland (PS) in subadult, 70 proteins associated with digestion, degradation, and secretion processes were identified as differentially expressed throughout the 24-hour cycle. These proteins correspond mainly to peptidases and hydrolases, although lipases were also identified. The heat map indicates that the presence of these proteins is time dependent, showing strong activation at 12:00 h, when preparation for the digestion of ingested food is likely taking place. This pattern coincides with the highest expression levels observed in the digestive gland (DG) between 08:00 and 16:00 h. Then, a gradual decrease is observed towards 16:00 h, followed by downregulation between 04:00 and 08:00 h. The comparison of the digestive gland (DG) proteome between juvenile and subadult specimens revealed differences in the temporal expression patterns of proteins involved in degradation, glucose metabolism, and digestion. In subadults, these proteins showed higher expression between 08:00 and 12:00 h, followed by downregulation that reached its minimum at 00:00 h. In contrast, juveniles exhibited less pronounced variations in the expression of proteins associated with degradation and glucose metabolism throughout the 24-hour cycle; however, digestive proteins showed a peak in expression at 04:00 h, a pattern that was not observed in subadults. Overall, the proteomic results obtained from the digestive gland (DG) and posterior salivary gland (PSG) of subadult and juvenile specimens demonstrate a marked temporal regulation of protein expression, associated with the existence of daily rhythms governing metabolic, digestive, energetic, and developmental processes. These rhythms are closely linked to the light/dark cycle.

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TRANSCRIPTOMIC RESPONSES TO SUBLETHAL EXPOSURE TO PHBV BIOPLASTICS IN *Artemia franciscana*

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Introduction

Bioplastics are increasingly promoted as sustainable alternatives to conventional fossil based plastics due to their biodegradability and biological origin. Among them, polyhydroxybutyrate covalerate (PHBV), a member of the polyhydroxyalkanoates (PHAs) family, is widely considered environmentally friendly. However, its biological effects on aquatic organisms at the molecular level remain poorly understood. This study investigated whether PHBV microparticles (~7.2 μm) are biologically inert by examining transcriptomic responses in the crustacean *Artemia franciscana*, which is widely used as a model organism in aquatic ecotoxicology research in marine and saline environments.

Materials and Methods

Instar II-III nauplii at a density of 0.5 individuals mL^{-1} were exposed for 14 days to two PHBV concentrations (low: 25 mg L^{-1} ; high: 100 mg L^{-1}) under controlled conditions in thermostatic chamber ($25 \pm 0.5^\circ\text{C}$ and 14:10 h light/dark photoperiod), and fed with fresh microalgae *Tetraselmis suecica* (20×10^4 cells mL^{-1} during the first 3 days, and 40×10^4 cells mL^{-1} for the remainder of the assay). Two controls were included, CTRL(+) fed with microalgae as food source without PHBV, and CTRL(−) group fed with microalgae and exposed to low concentrations of polystyrene (PS, 25 mg L^{-1}). For each treatment and the controls, five biological replicates were established. Data on survival was recorded during the total medium renewal (twice a week). Samples collected after 14 days of exposure were preserved in RNAlater and stored at -80°C until RNA extraction. Total RNA was isolated using the RNeasy Mini Kit, quantified, barcoded and prepared for Illumina sequencing. For the transcriptomic analysis, all groups were compared against the transcriptomic profile of the CTRL(+) group. Differentially expressed genes (DEGs) were identified and examined through enriched terms (GO) and KEGG pathways to determine affected biological pathways.

Results and discussion

After 14 days of exposure, PHBV concentrations did not significantly affect brine shrimp survival, which remained above 72% in all treatments, including CTRL(+).

Transcriptomic analysis revealed concentration-dependent responses to PHBV exposure. Bioplastic exposure mainly downregulated genes involved in lipid metabolism, carbohydrate transport and metabolism, and immune defense pathways, suggesting impaired energy acquisition and physiological performance. In contrast, upregulated DEGs were primarily associated with oxidative stress response, redox homeostasis, and detoxification pathways, including xenobiotic metabolism, glutathione-related processes, and oxidoreductase activity. Additionally, genes involved in lipid and energy metabolism were enriched among the upregulated DEGs, suggesting increased metabolic demands and the activation of protective mechanisms in response to PHBV exposure. Overall, these results indicate a coordinated physiological response to PHBV, characterized by metabolic suppression and activation of defense mechanisms. The upregulation of detoxification and antioxidant pathways suggests that PHBV is recognized as a stressor at the molecular level, while the downregulation of metabolic and transport processes may reflect an energy reallocation strategy under stress conditions. These findings demonstrate that PHBV is not biologically inert and can induce significant sublethal molecular effects in aquatic organisms.

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EVALUATION OF *Lagocephalus sceleratus* FISHMEAL AS A SUSTAINABLE ALTERNATIVE PROTEIN SOURCE IN EUROPEAN SEA BASS (*Dicentrarchus labrax*) DIETS: EFFECTS ON GROWTH, NUTRIENT DIGESTIBILITY, GUT MICROBIOTA AND IMMUNE STATUS

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Introduction

Alien species, also referred to as non-indigenous species, have rapidly colonised the Eastern Mediterranean basin. Those exhibiting invasive potential have caused significant impacts on biodiversity, ecosystem services, and human well-being. Among them, *Lagocephalus sceleratus* (Gmelin, 1789) is a highly opportunistic species responsible for substantial damage to fishing gear, catches, and local biodiversity, while also posing serious risks to human health. This species is highly toxic and currently holds no economic value in the Mediterranean market, as it is prohibited from commercialisation under EU legislation. Its continued spread is facilitated by the absence of natural predators, combined with high growth and reproductive rates. This situation highlights the urgent need for alternative management strategies for invasive species. In parallel, the rapid expansion of aquaculture has increased the demand for aquafeeds, while the overexploitation of marine resources for fishmeal production necessitates the identification of sustainable alternative protein sources. The present study aimed to evaluate the potential use of fishmeal derived from *Lagocephalus sceleratus* as a partial or total replacement for conventional fishmeal in diets for European sea bass (*Dicentrarchus labrax*).

Materials and methods

Specimens of *Lagocephalus sceleratus* were processed into fishmeal through cooking, pressing, drying, and grinding. The resulting meal was subjected to two thermal treatments (160°C and 210°C) to reduce tetrodotoxin (TTX) levels, producing lower temperature (T1) and higher temperature (T2) variants. Five experimental diets were formulated, in which conventional fishmeal was replaced totally with unprocessed *Lagocephalus sceleratus* meal (UnP) or thermally treated variants (T1 and T2), including one diet with partial substitution by T1 (Combo). The UnP diet served as a negative control to evaluate the tolerance of European sea bass to TTX. European sea bass with an initial body weight of 39 g were randomly distributed into 15 tanks, with each dietary treatment tested in triplicate. Fish were reared in an open-flow system under controlled water quality conditions for 106 days (20°C, >90% O₂ saturation) and were fed the experimental diets to apparent satiation twice daily. Faeces were collected daily for the last 15 d of the trial to determine apparent digestibility coefficients (ADC). Digestibility, whole-body composition, intestinal trypsin activity, gut microbiota, immune status, and histological analyses were performed to comprehensively evaluate the effects of the experimental diets. TTX levels were assessed in liver, muscle, and whole-body samples using LC–MS/MS (LOD: 0.0005 mg/kg). Microbial community analysis was conducted on gut DNA using 16S rRNA gene amplicon sequencing (Illumina MiSeq, 2 × 300 bp, V3–V4 region). Sequences were processed in Mothur (v1.48.0), with OTUs defined at 97% similarity and taxonomically assigned against the SILVA database (release 138.1).

Results and discussion

Most production parameters differed significantly among treatments. Specific growth rate (SGR) and feed conversion ratio (FCR) were significantly improved in fish fed the UnP diet. The reduced performance observed in fish fed T1 and T2 diets were likely associated with a decline in the nutritional quality of the meals, as high processing temperatures may promote lipid oxidation, degradation of highly unsaturated fatty acids, and overall nutrient loss (Phung et al., 2020). The highest protein digestibility was observed in fish fed the UnP diet, followed by the Control group. Intermediate values were recorded in the Combo group, while fish fed thermally processed fishmeals (T1 and T2) exhibited markedly reduced protein digestibility, consistent with their lower final body weight. Similarly high lipid digestibility above 95% was observed in the UnP and Control diets. The lowest lipid digestibility was recorded in fish fed the high-temperature processed fish meal (T2). Significant differences were observed in whole-body lipid content. Fish fed the T2 diet exhibited the highest lipid

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levels compared to the Control and UnP groups. This outcome is likely associated with the markedly reduced protein digestibility observed in this group, which may have led to increased feed intake to meet energy demands, resulting in greater lipid deposition. Previous studies have associated reduced digestive enzyme activity, particularly trypsin, with impaired growth performance and feed utilisation (Murashita et al., 2018). In the present study, intestinal trypsin activity was highest in fish fed the Combo, UnP and Control diets. In contrast, the lowest trypsin activity was observed in fish fed the T2 diet, supporting the reduced performance observed in this group. Moreover, no significant differences were observed in gut bacterial communities among dietary groups. The dominant bacterial phyla – Pseudomonadota, Actinomycetota, Bacillota and Bacteroidota – commonly reported in the fish gut microbiomes, were consistently present across all dietary treatments, with only minor variations in relative abundance. Serum trypsin inhibitor activity, an indicator of immune resistance to the pathogens evasion process, was highest in fish fed the Unp, Combo, and T2 diets, with no significant differences compared to the Control group. The lowest values were observed in the T1 group. Lysozyme activity, reflecting antibacterial capacity against Gram-positive bacteria, was significantly higher in fish fed the UnP diet and the Combo diet compared to the Control and T2 groups. Concerning the antibacterial activity against Gram-negative bacteria, T1 and Combo diets showed significantly higher values than the Control diet. No histological differences were observed in the liver among experimental groups. Moderate hepatocellular vacuolation, indicative of lipid accumulation (steatosis), was consistently observed. Similarly, no morphological differences were detected in either the anterior or posterior intestine. All groups exhibited intact epithelial structure, with no signs of hypertrophy or hyperplasia. Complete replacement of conventional fishmeal with unprocessed *Lagocephalus sceleratus* meal (UnP) resulted in superior growth, nutrient digestibility, immune response, and digestive enzyme activity. Partial substitution with lower temperature treated meal T1 (15%) achieved comparable performance. Thermal processing reduced the nutritional quality of the meals, as evidenced *in vivo* by decreased growth, protein digestibility, and enzyme activity in fish fed exclusively treated diets. Importantly, no TTX accumulation was detected in any tissue. These findings support the potential of valorisation of *L. sceleratus* as a sustainable alternative protein source, contributing to both invasive species management and aquaculture sustainability. However, further validation under long-term and large-scale production conditions is required.

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NEW PROTOCOL FOR THE INDUCED SPAWNING OF PIKEPERCH (*Sander lucioperca*): VIDEO CAMERA-BASED OVULATION DETECTION

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Introduction

The pikeperch (*Sander lucioperca*) is a well-known species in Europe, with established protocols for its induced reproduction. However, these methods are highly labor-intensive, requiring repetitive female handling. This procedure causes physiological stress, which may negatively affect progeny development (Rasal et al., 2024) and increase female mortality rates (Rónyai, 2007). Consequently, a critical area of research in modern aquaculture is the identification and application of objective spawning indicators that allow for a more accurate and less invasive recognition of the ovulation. In light of the above, the present study aims to detect and utilize behavioral indicator that enables reliable ovulation detection using camera surveillance, further establishing an animal-friendly and labor-reducing protocol for pikeperch artificial reproduction.

Materials and methods

In January 2025, six breeding pairs were hormonally induced using a salmon gonadotropin-releasing hormone analogue (sGnRHa) under a warming thermal regime (Ljuboratić et al., 2021). The pairs were housed in two 4 m³ tanks, each partitioned into three spawning chambers, resulting in six individual breeding units. Each unit was equipped with a spawning substrate and monitored by an overhead camera. Environmental conditions were controlled via an underwater lighting system set at a 12:12 light:dark (LD) photoperiod. Once the oocyte reached germinal vesicle breakdown (GVBD), the lighting was switched to continuous illumination (24:0 LD) to ensure optimal image quality for the camera systems.

In February 2025, in addition to the nesting pairs (NEST), an additional tank was set up as a control group (TANK), following the common protocol for pikeperch, where a mesh divider separated fish by sex (Ljuboratić et al., 2025). Hormonal, thermal, and photoperiod regimes were identical to those used for the previous spawning batch. Starting from the sixth day post-stimulation, oocyte biopsy was conducted daily in all females. Upon reaching the GVBD stage, the female's genital papilla was sutured (Žarski et al., 2017). Further on, ovulation in the TANK group was monitored every 6 h; in contrast, ovulation in the NEST group was monitored only after the detection of a previously defined ethological indicator. NEST females were stripped one hour after the spawning behavior was observed, while the stripping of the TANK group was performed at the moment the ovulation (egg release) was witnessed upon abdominal massage. After fertilization, egg stickiness was removed, and eggs from each female were incubated separately in Zuger jars for evaluation of the egg quality.

Results and Discussion

In the first batch, spawning was observed in all six pairs on the 8th and 9th days after hormonal stimulation. The ethological indicator of spawning was successfully recorded in all fish. In the subsequent trial, the identified indicator led to the precise timing of stripping. In the second experimental cycle, eggs were freshly stripped in all cases based on the deformation observed during the cortical reaction (Nynca et al., 2024). No significant differences were observed between the nesting and control groups regarding latency time or egg quality. However, a statistical trend ($P = 0.086$) was identified concerning oil globule fragmentation, being higher in the TANK group.

The fact that high egg quality was achieved in all cases using the behavioral indicator suggests that this non-invasive monitoring is as effective as the common ovulation check that requires excessive handling. Recognizing the specific ethological sign before handling the sutured females, the optimal stripping time is ensured, avoiding both early egg stripping and over-ripening of the eggs (Nynca et al., 2024). Furthermore, the success of this monitoring approach opens new perspectives for automated hatchery management. The image processing data collected during the trials will serve as a basis for developing an Artificial Intelligence (AI)- based alarm system, that will automatically detect the real-time onset of spawning. This integration of machine learning algorithms will eliminate the need for manual video surveillance, increasing the efficiency of pikeperch reproduction management.

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AQUACULTURE AS A POLICY LEVER FOR SUSTAINABLE FOOD SYSTEMS: A FOOD-ENVIRONMENT FRAMEWORK FROM THE SASPA PROJECT IN ITALY

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Introduction

Aquaculture is increasingly recognised as a strategic component of sustainable food systems because it could supply nutritious aquatic foods, reduce dependence on imports, create coastal employment and contribute to the EU blue transition when supported by appropriate governance and innovation (European Commission, 2021; FAO, 2024). Yet aquaculture development remains constrained not only by production bottlenecks, but also by weak social acceptability, fragmented policy coordination, and consumer uncertainty about origin, quality, price and sustainability (Masi et al., 2022; Vecchio et al., 2024; Perez Agundez et al., 2025). The food-environment perspective helps address these limits by focusing on how availability, affordability, information, trust and desirability shape food choices (Herforth and Ahmed, 2015; Kennedy et al., 2023). This paper presents preliminary evidence from the SASPA project in Italy and proposes a policy-oriented framework to position aquaculture within sustainable food systems, using the Mediterranean Diet as a strategic narrative capable of reconnecting production, consumption and territorial value creation (Capone, 2024). SASPA is a national multi-stakeholder action-research initiative jointly developed by MASAF and CIHEAM Bari. The analysis combines: (i) a review of EU and international policy frameworks on aquaculture and sustainable food systems; (ii) a review of the literature on food environments, consumer behaviour, social acceptability and aquaculture innovation; and (iii) the first outputs of the SASPA participatory process, including the 2025 stakeholder webinar, the position paper and the action-priority matrix developed with institutions, producer organisations, researchers and food-system intermediaries. The analytical objective is not to evaluate farm performance per se, but to identify the policy domains through which aquaculture can become more economically viable, socially legitimate and nutritionally relevant within Italian food environments.

Results and Conclusion

Preliminary results indicate that a food-environment lens can shift aquaculture policy from a narrow supply-side approach to a broader framework of demand formation and public value creation. Three interdependent policy domains emerge. First, access: sustainable aquaculture requires not only production support, but also better physical and economic access through logistics, distribution, product diversification and public procurement, especially in schools, hospitals and collective catering. This is particularly relevant in Mediterranean contexts, where contemporary lifestyles often weaken the regular consumption of aquatic foods despite their nutritional relevance (Meybeck et al., 2024). Second, trust: traceability, transparency and credible information are central economic variables because they reduce consumer uncertainty and support willingness to pay for national aquaculture products. Existing evidence on farmed fish consumption in Mediterranean countries shows that origin, freshness, quality cues and sustainability perceptions strongly affect demand (Masi et al., 2022). In parallel, current research on Italian aquaculture policy networks suggests that innovation uptake depends on better alignment between institutions, sectoral actors and society, rather than on technology transfer alone (Vecchio et al., 2024). This broader framing is consistent with recent work on circular economy pathways in aquaculture, which stresses the need for systemic innovation across value chains, resource use and governance arrangements (Masi et al., 2024). Third, desirability: aquaculture products need to be embedded in cultural and dietary narratives that make them socially meaningful, convenient and desirable. Here the Mediterranean Diet can operate as a policy bridge between health, environment, heritage and market development (Serra-Majem et al., 2020). Communication, culinary mediation, digital information tools and nutrition education therefore become policy instruments, not ancillary actions. This is consistent with recent calls to treat aquatic foods as central, rather than peripheral, within food and nutrition policy (Bennett et al., 2021; Kennedy et al., 2023). Overall, SASPA suggests that aquaculture should be framed as a strategic infrastructure of sustainable food systems. In policy terms, this implies integrating aquaculture with consumer policy, public health, education, territorial development and digitalisation, while addressing the social acceptability issues that continue to limit sector expansion in Europe (European Commission, 2021; Perez Agundez et al., 2025).

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Acknowledgment

This abstract presents preliminary results from SASPA (2025-2027), developed within the collaboration between MASAF and CIHEAM Bari.

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WATER MOLD (*Saprolegnia parasitica*) INFECTIONS IN AQUACULTURE PREDICTED BY MACHINE LEARNING FROM WATER QUALITY DATA

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Introduction

Saprolegniosis caused by *Saprolegnia* spp. oomycetes, also referred to as water mold, is a serious threat to fish farming and wild fish and other aquatic animals in fresh water environments. It is one of the major obstacles in fish production and even threatens some highly endangered species (Eronen et al. 2024). Currently, it is still inadequately understood which abiotic and biotic factors in water quality or other conditions in nature and at fish farms influence active saprolegniosis incidences (Pavić et al. 2022). Stressful conditions in aquaculture farms (temperature shock, additional infections, overcrowding, injuries) can weaken the fish's immune system and increase its susceptibility to *S. parasitica*. However, it is still unclear how these interactions of different factors work (Lindholm-Lehto and Pylkkö 2024).

Materials and Methods

Using daily water-quality and fish mortality data from Natural Resources Institute Finland (Luke)'s Enonkoski aquaculture infrastructure (2024-2025) rearing endangered landlocked salmon (*Salmo salar* m. *sebago*) for re-stocking, we developed an interpretable Random Forest model that predicts infection occurrence. Infection densities were estimated with a walk-forward two-part hurdle generalized additive model (GAM), and past-only lag and rolling features (1–28 days) were derived from water-quality variables.

Results

A weighted Random Forest using the 50 top-ranked features achieved strong probabilistic performance (Brier = 0.096 ± 0.037 ; LogLoss = 0.341 ± 0.194 ; ROC-AUC = 0.989). Calibrated forecasts correctly identified the start of a new infection period, with late-stage lethal infection probability rising several days before the first detected cases. SHapley Additive exPlanations (SHAP) and permutation analyses revealed that infection risk was highest under oxygen-rich, moderately warm (12–17°C), and stable organic matter conditions, whereas high turbidity and strong UV254 (aromatic organic matter) variability reduced risk.

Discussion

An interpretable Random Forest model was able to quantify how water-quality dynamics over the preceding month predict late-stage lethal *S. parasitica* infections in an aquaculture system. By combining daily water-quality measurements with infection observations and model-based label estimation, we identified key environmental precursors and lag structures preceding infection events. Our goal was not only to achieve accurate prediction, but to provide mechanistic insight into the environmental context favouring *S. parasitica* growth and to demonstrate a transparent modelling framework for future early-warning applications.

Acknowledgments

The experiment was funded by the European Union through the European regional Fund (EAKR) The Finland 2021–2027 innovation and skills in Finland program, municipality of Enonkoski, and Hanka-Taimen Oy. We would like to thank the staff at the Natural Resources Institute Finland, Enonkoski.

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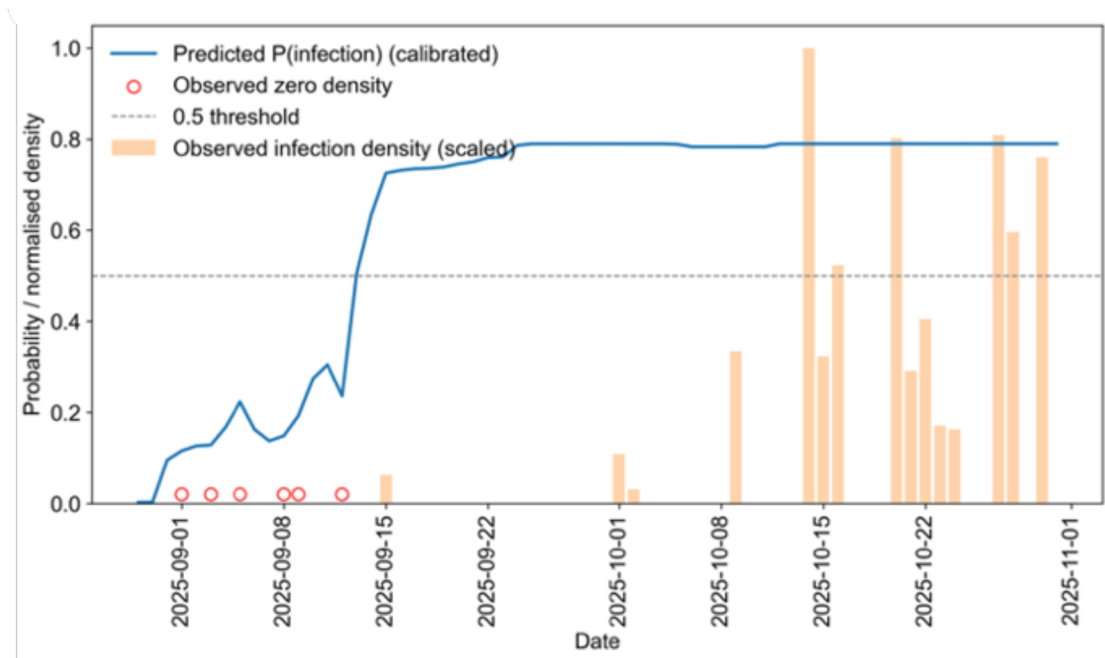


Figure 1. Infection probability forecasts for an independent test period (September–October 2025). The blue line shows predicted infection probability, orange bars indicate observed infection density (normalised for visual comparison), and open red circles mark days with zero observed infection. The dashed horizontal line denotes the operational decision threshold (0.5). Infection probability begins to rise approximately 15 days before the first observed infection.

A PHYSIOLOGICALLY RELEVANT IN VITRO MODEL OF RAINBOW TROUT (*Oncorhynchus mykiss*) INTESTINAL IMMUNITY AND ITS INFLAMMATORY RESPONSIVENESS

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Introduction

The rainbow trout (*Oncorhynchus mykiss*) intestine is organized into two main functional districts, the proximal and distal regions, each contributing differently to nutrient absorption and mucosal immunity. Like other teleosts, trout lack organized GALT structures, resulting in a diffuse intestinal immune system largely coordinated by enterocytes[1] .

To reproduce this complexity *in vitro*, we established two epithelial cell lines derived from the proximal and distal intestine [2]. Building on these models, and on culture conditions already described in our previous work [3,4], we propose here a functional reinterpretation of conventional *in vitro* settings, defining two complementary epithelial states: a *niche-like module*, associated with proliferation and stemness, and a *fold-like module*, associated with enterocyte differentiation. Importantly, these modules represent a biologically grounded framework for interpreting epithelial phenotypes emerging under standard conditions.

Using these established systems, we explored their intrinsic immune competence under physiological, non-inflammatory conditions. We assessed the spatial distribution of IL8, IL6, IL1 β , and TNF α through *in situ* hybridization, comparing *in vitro* results with the *in vivo* intestine to evaluate the physiological relevance of this interpretative model. Finally, we tested their responsiveness to an inflammatory stimulus (LPS) by monitoring the transcriptional activation of pro-inflammatory cytokines.

Results and discussion

In vivo, all four cytokines were detected at low levels and were mainly restricted to the intestinal mucosa. TNF α and IL8 were the most abundant markers and the only ones showing occasional strongly positive cells, whereas IL6 and IL1 β were expressed only in rare cells. No major differences were observed between proximal and distal regions or between the stem cell niche and differentiated fold, indicating a relatively homogeneous distribution of the diffuse immune system along the trout gut.

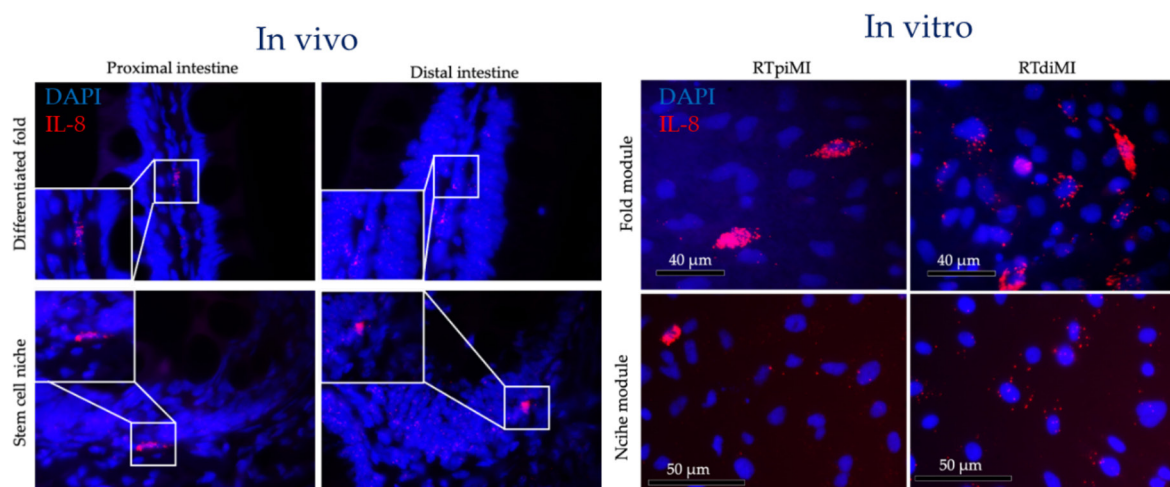


Figure 1: Representative *in situ* hybridization of IL-8 (red signal) in the rainbow trout intestine and in the respective *in vitro* models. The images include both proximal and distal regions and highlight the two functional epithelial units that characterize the trout gut: the stem cell niche and the differentiated fold. Corresponding *in vitro* images from the proximal and distal epithelial cell lines are shown for both the niche and fold modules. Nuclei are counterstained with DAPI (blue).

(Continued on next page)

In vitro, all four genes were consistently expressed in both epithelial cell lines and across the two functional modules, confirming that trout enterocytes retain intrinsic immune competence. As observed *in vivo*, IL8 and TNF α were the most highly expressed markers and showed occasional highly positive cells, suggesting the presence of a particularly responsive epithelial subset. Overall, the spatial and qualitative expression patterns closely mirrored the *in vivo* reference, supporting the physiological relevance of interpreting these culture conditions as functionally distinct epithelial states.

Upon LPS exposure, both modules showed activation of the inflammatory program, with upregulation of several pro-inflammatory cytokines. This response indicates that these epithelial systems not only preserve basal immune competence but also retain the ability to sense and respond to external stimuli. While further work is needed to dissect the specificity and magnitude of these responses, the results support the validity of this *in vitro* framework.

Taken together, these findings highlight that appropriate culture conditions can recapitulate distinct, physiologically meaningful epithelial states when interpreted within a coherent biological framework. This approach provides a practical and scalable platform for aquaculture applications, enabling the screening of feed ingredients and functional compounds, as well as the evaluation of their effects on intestinal immune regulation in a region-specific and physiologically relevant context.

Acknowledgment

This work was supported by NUTRIsim

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EARLY SEX PHENOTYPING IN EUROPEAN SEABASS (*Dicentrarchus labrax*): EVALUATION OF NON-INVASIVE ULTRASONOGRAPHY ACROSS DIFFERENT AGE GROUPS

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Introduction

European seabass industry is primarily supplied with juveniles originating from selective breeding programs. These programs are managed by private hatcheries, which bear the full operational and financial costs of all selection steps. Because sex determination in this species is polygenic and highly sensitive to environmental variations (Vandeputte et al., 2007) although suspected in several species, is thought to be evolutionarily unstable and has been proven in very few cases. In the European sea bass, temperature is known to influence the sex ratio. We set up a factorial mating, producing 5.893 individuals from 253 full-sib families, all reared in a single batch to avoid any between-families environmental effects. The proportion of females in the offspring was 18.3%, with a large variation between families. Interpreting sex as a threshold trait, the heritability estimate was 0.62 ± 0.12 . The observed distribution of family sex ratios was in accordance with a polygenic model or with a four-sex-factors system with environmental variance and could not be explained by any genetic model without environmental variance. We showed that there was a positive genetic correlation between weight and sex ($r_A = 0.50 \pm 0.09$, the sex ratio among selection candidates varies considerably and is difficult to phenotype the sex of individual fish *in vivo* before the age of three, as the species exhibits no secondary sex traits. When larvae are reared under standard hatchery conditions, the sex ratio is heavily biased toward males; however, mass selection tends to favor females due to their higher growth rate (Geffroy et al., 2021). This constraint forces hatcheries to rear an excessively large number of prospective breeders for three years just to guarantee a balanced sex ratio among their final breeding stock.

Materials and Methods

This study investigates the conditions under which ultrasonography (echography) can enable early non-invasive sex phenotyping in seabass. To this end, four batches of European seabass were evaluated: aged 10, 22, 34, and 48 months, with mean weights of 118 g, 330 g, 614 g, and 3000 g, respectively. Sex phenotyping was performed using two different ultrasound devices: an ExaPad equipped with an LC1428V probe (IMV Technologies) and a Clarius L7 Vet (Clarius). To mimic commercial hatchery operations, the three younger batches were reared under standard grow-out conditions at a constant temperature of 21°C. Conversely, the oldest batch was maintained for over a year under maturation conditions, where water temperatures followed the natural seasonal variations of the Gulf of Lion in the Mediterranean Sea (13°C in winter to 25°C in summer). Under veterinary supervision, five operators used the devices to determine the sex of 151, 250, 128, and 110 fish (from the youngest to the oldest batch, respectively). Ultrasound examinations were performed on anaesthetised fish. For each fish, the probe was manipulated by one random operator, and the sex prediction was established independently by 2 to 5 observers, resulting in a total of 1,910 predictions. The true sex of the fish was subsequently validated by macroscopic observation of the gonads post-mortem for the three younger batches, and by gamete sampling for the oldest batch.

Results

The proportion of males in the batches was 69.6%, 56.9%, 51.5%, and 28.2% from the youngest to the oldest, respectively. Diagnostic accuracy calculated as the percentage of correctly identified fish out of the total evaluated, yielded the following results: 57%, 81%, 87%, and 96% for the 10-, 22-, 34-, and 48-month-old batches, respectively. The accuracy for the 10-month-old batch was significantly lower than that of the other groups ($p = 2.17 \times 10^{-8}$). Linear model analysis revealed that males were more accurately predicted in the 22- and 34-month-old batches ($p = 2 \times 10^{-16}$ and $p = 0.00883$, respectively). Furthermore, the operator manipulating the probe did not achieve higher predictive accuracy than the non-manipulating observers.

Discussion

In conclusion, this study demonstrates that it is not possible to reliably predict the sex of 10-month-old fish kept under standard grow-out conditions. However, non-invasive ultrasonography proves to be a highly effective tool for adjusting unbalanced sex ratios in fish aged 2 to 3 years kept in growing condition.

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OVERCOMING KEY BOTTLENECKS IN SEA URCHIN AQUACULTURE: LESSONS FROM CLOSED LIFE-CYCLE AND ROE-ENHANCEMENT CULTURE OF AN AUSTRALIAN URCHIN

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Across Europe, sea urchins are simultaneously too abundant and too scarce: overabundance drives the expansion of barrens in some regions, while overexploitation has depleted fishery stocks in others. How do we restore balance? That is where aquaculture comes in. Two aquaculture approaches are emerging: capture-based roe-enhancement of overabundant species and hatchery-based restocking of depleted populations. However, several bottlenecks exist that limit the economic viability of both approaches.

Using the Australian purple sea urchin (*Heliocidaris erythrogramma*) as a model, we integrate findings from broodstock conditioning, juvenile feeding, and commercial-scale roe-enhancement to identify key drivers of production success. For closed life-cycle culture, we successfully developed effective formulated broodstock diets and identified suitable diets across the juvenile grow-out phase. For roe-enhancement, we created “gold-standard” collection and culture techniques that ensure high survival rates (>90%) and produced roe exceeding market standards in both quality and size at commercial scale.

Here, we summarise the results of three studies and discuss how these findings can inform the emerging roe-enhancement industry of the overabundant *Strongylocentrotus droebachiensis* in Norway, and restocking programs for *Paracentrotus lividus* in Southern-Europe.

Artemia*: NO FOOD LEFT BEHIND - RECYCLING SIDE-STREAMS FROM THE FOOD INDUSTRY FOR VALORIZATION AND ALTERNATIVE PROTEIN PRODUCTION USING THE NON-SELECTIVE FILTER FEEDER *Artemia franciscana

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Introduction

Body text (Aptos, 10 pt, single spacing, justified). This may be an introduction and/or an explanation of the methodology. Write abbreviations/acronyms in full for first time use. The food industry generates substantial quantities of side-streams that often possess limited economic value and may incur disposal or recycling costs. At the same time, the aquaculture sector faces increasing pressure to identify sustainable and locally produced protein sources that can reduce reliance on conventional feed ingredients. The VAL@rtemia project explores the potential of converting food-industry side-streams into valuable biomass through cultivation of the brine shrimp *Artemia franciscana*. As a non-selective filter feeder, *Artemia* can utilize a wide range of particulate feed sources, making it a promising candidate for circular bioeconomy applications. The project aims to valorize food-industry side-streams, establish large-scale *Artemia* production systems based on these streams and evaluate their economic feasibility.

Methodology

A range of side-streams originating from the Flemish food industry is being screened and evaluated for suitability as feed for *Artemia franciscana*. To date, five side-streams have been selected for evaluation: coffee grounds, sugar water, lactose serum, beer yeast and grey starch. Depending on their physical and chemical characteristics, side-streams are subjected to pre-treatment steps including blending, grinding, dilution and pH adjustment to improve their suitability for cultivation. Different concentrations of each side-stream are assessed to determine their impact on *Artemia* performance and survival. Promising candidates are subsequently evaluated in recirculating aquaculture systems (RAS) with *Artemia*. In parallel, market opportunities, legal considerations and upscaling potential are assessed to support future implementation at commercial scale.

First Results

Initial screening activities indicate that side-stream characteristics strongly influence their suitability for *Artemia* cultivation. The evaluated side-streams differ considerably in their composition and therefore require varying levels of pre-treatment before use as feed. Among the side-streams tested so far, coffee grounds and sugar water emerged as particularly promising candidates. These streams required relatively limited pre-treatment while maintaining *Artemia* survival rates exceeding 90% at tested concentrations ranging from 0.1% to 5%. The remaining side-streams continue to be evaluated as part of the ongoing screening process.

These preliminary findings support the potential of selected food-industry side-streams as low-cost substrates for *Artemia* biomass production. By converting low-value residual streams into aquatic biomass, the process contributes to nutrient recycling and improved resource efficiency. Furthermore, production of locally sourced *Artemia* biomass may strengthen the sustainability of aquaculture supply chains by providing an alternative protein source.

Market exploration has identified opportunities in aquafeeds, where *Artemia* biomass could serve as an alternative protein source or feed additive, potentially reducing the dependence on fishmeal-based formulations. Additional opportunities were identified in the pet food sector, particularly for hydrolysed protein products, where demand for novel and sustainable protein ingredients is increasing.

Conclusion and Next Steps

Initial screening of five side-streams has identified promising candidates for further development, with coffee grounds and sugar water showing encouraging results. Ongoing work will focus on optimization of cultivation protocols, evaluation of additional side-streams, operation in recirculating aquaculture systems, assessment of economic feasibility and identification of viable market applications. By combining side-stream valorization with sustainable protein production, the project aims to contribute to more circular and resource-efficient food systems.

Acknowledgment

This work is conducted within the VAL@rtemia project, funded by the European Union (project grant nr. 25-2.1.3-10-DIV).

CIRCULAR NUTRIENT RECOVERY FROM AQUACULTURE SLUDGE FOR SEAWEED PRODUCTION USING A NOVEL MINERALIZATION REACTOR

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Introduction

The Project Circular Systemic Water-Energy-Food-Ecosystems Nexus (CisWEFE-NEX) aims to implement a circular, systemic Water-Energy-Food-Ecosystem (WEFE) nexus for regions facing severe water stress by integrating sustainable technologies for improved resource efficiency. Within recirculating aquaculture systems (RAS), drum filter sludge represents a concentrated side stream rich in phosphorus, minerals and organic matter, yet its valorisation remains limited. Upflow Anaerobic Sludge Blanket (UASB) reactors are recognised as highrate anaerobic treatment systems that can operate at reduced hydraulic retention times and support energy recovery, and have been proposed as key units in circular wastewater treatment and nutrient recycling strategies (Mainardis et al., 2020).

Materials and Methods

Landing Aquaculture developed a mineralisation reactor based on UASB (Upflow Anaerobic Sludge Blanket) technology for treatment of drum filter sludge from RAS. Sludge is collected in a sump and pumped into the bottom of the reactor through lowturbulence inlets, where a sludge blanket forms in the lower half to twothirds of the vessel. Internal circulation maintains the blanket in suspension, while pH and Redox are monitored and adjusted to support stable acidogenic conditions. Under this operational mode, solids are retained for approximately 4 days, promoting volatile fatty acid formation, denitrification, and phosphorus and mineral release. Clarified supernatant exits through submerged outlet pipes and can be further polished before storage or reuse. This process concept aligns with key UASB design considerations related to sludge retention, hydrodynamics, and process control reported in recent reviews (Mainardis et al., 2020).

Discussion

The reactor concept is intended to couple sludge treatment from aquaculture production with nutrient recovery for seaweed production within the CisWEFE-NEX circular framework. Anaerobic treatment of retained sludge enables rapid nutrient solubilisation while supporting simultaneous denitrification, thereby improving the suitability of the treated stream for reuse within aquaculture operations. Compared with conventional waste handling, this approach supports the conversion of a residual sludge fraction into a nutrientrich liquid stream that may be directed toward biomass production. Reviews of aquaculture waste nutrient treatment highlight both the potential and constraints of valorising waste nutrients in pond and recirculating systems, and emphasise the need for integrated, sitespecific solutions (Tabrett et al., 2024). In addition, operation at longer retention times could allow methanogenic conversion and energy recovery, expanding the role of the reactor within integrated WEFE systems (Mainardis et al., 2020).

Conclusion

UASBbased sludge mineralisation provides a compact and flexible strategy for converting RAS sludge into a nutrient source for seaweed production. By combining nutrient solubilisation, denitrification, and the possibility of biogas generation, the system supports a circular aquaculture model aligned with the CisWEFE-NEX approach. Further validation in integrated aquaculture-seaweed systems will help define its full potential for lowfootprint biomass production and nutrient reuse.

Acknowledgment

This work is being conducted under the CisWEFE-NEX project, funded by Horizon Europe (Grant agreement No. 101135062).

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IDENTIFYING QTLs FOR RESISTANCE TO RAINBOW TROUT FRY SYNDROME IN AN ORGANIC LINE OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Introduction

Antibiotic use is restricted in organic aquaculture, increasing the need for sustainable strategies to enhance disease resistance. Selective breeding represents a promising approach to improve host disease resistance, and genomic tools such as quantitative trait locus (QTL) mapping have been widely used to identify genetic regions associated with disease resistance in aquaculture species. A globally prevalent and devastating disease in rainbow trout (*Oncorhynchus mykiss*) production is Rainbow Trout Fry Syndrome (RTFS), caused by *Flavobacterium psychrophilum*. This bacterial disease can result in high mortalities, particularly among fry and juvenile fish. Its impact on production can be substantial, leading to significant economic losses in commercial aquaculture. Currently, no commercial vaccine against RTFS is available. Control of the disease typically relies on antibiotic treatments, however their use raises environmental concerns and resistant strains of *F. psychrophilum* to various types of antibiotics have been reported (Barnes & Brown, 2011). The impact of RTFS is particularly concerning in organic aquaculture, where the number and type of treatments are limited, hence requiring alternative disease management strategies. Several QTLs for RTFS resistance were already identified in conventional strains of rainbow trout (Fraslin et al., 2019; Mathiessen et al., 2023; Palti, Vallejo, et al., 2015). However, it remains unknown whether these QTLs are present or effective in organic strains, which may differ in genetic architecture from conventional strains due to distinct selection pressures. Addressing this knowledge gap is critical for the development of targeted breeding programs in organic aquaculture. Therefore, this study aims to identify QTL associated with RTFS resistance in an organic rainbow trout line.

Materials and Methods

In this study, we sampled rainbow trout at an organic farm in Denmark during a natural outbreak of RTFS. The population consisted of 210 full- and half-sib families, produced in April 2025. For each family, 18 fish were randomly distributed across three tanks (six fish from each family per tank). The RTFS outbreak began in mid-September 2025 and ended in early November 2025, with a duration of 47 days in total. Throughout the outbreak, mortalities were sampled daily. Surviving fish were sampled after the disease outbreak. DNA was extracted from tissue samples and genotyped using the 57K SNP Axiom Trout Genotyping Array (Palti, Gao, et al., 2015) at a commercial laboratory (Eurofins, Galten, Denmark). Quality control on SNP level was performed using PLINK v1.9. SNPs were excluded based on the following criteria: (i) call rate <90%, (ii) deviation from Hardy–Weinberg equilibrium ($P < 1 \times 10^{-5}$), and (iii) minor allele frequency (MAF) <0.05. We performed GWAS analysis for RTFS resistance defined as a binary trait (survival: 0/1). An association analysis was conducted using the mixed linear model association (MLMA) framework with a leave-one-chromosome-out (LOCO) approach, as implemented in GCTA. The model used was:

$$y = a + bx + g^- + e$$

where y is the corrected phenotype, a is the mean term, b is the additive effect (fixed effect) of the candidate SNP to be tested for association, x is the SNP genotype indicator variable coded as 0,1 or 2, g^- is the polygenic effect (random effect) of all SNPs except those on the chromosome where the tested SNP is located and e is the residual. A SNP was considered significant when exceeding a Bonferroni corrected threshold of $P < 2.01 \times 10^{-6}$ ($0.05/24,879$).

Results and Discussion

In total, 295 survivors and 1,195 mortalities were sampled. After quality control, the final dataset comprised 1,490 samples and 24,879 SNPs. Significant SNPs associated with RTFS resistance were detected on chromosomes 13 and 25 (Fig. 1). The lead SNPs on chromosomes 13 and 25 explained 4% and 10% of the phenotypic variance, respectively. Significant SNPs on chromosome 25 have been reported previously (Mathiessen et al., 2023; Palti, Vallejo, et al., 2015), whereas chromosome 13 has not previously been associated with RTFS resistance.

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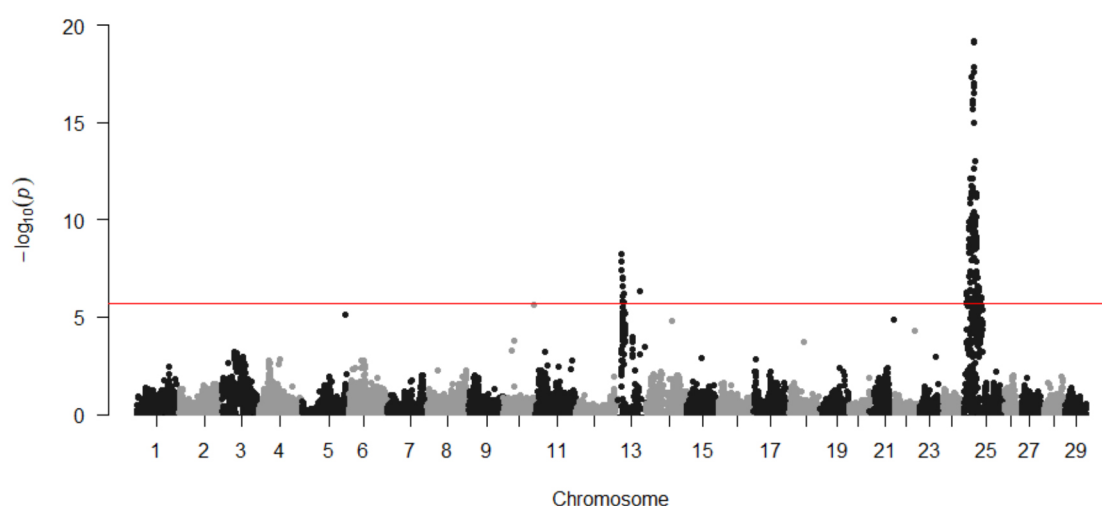


Figure 1. Results of GWAS for RTFS resistance as binary trait (survival: 0/1), with a Bonferroni-corrected significance threshold of $p < 2.01 \times 10^{-6}$.

This study demonstrates that RTFS resistance is influenced by multiple loci explaining a relatively high proportion of the phenotypic variance. This indicates that these SNP markers can potentially be used to improve RTFS resistance in an organic rainbow trout breeding population. Because the sampling design reflected the natural infection route in an organic production system, the results can be directly applied to selective breeding programs targeting RTFS resistance in organically farmed rainbow trout.

Acknowledgment

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PEANUT PEEL AS A FUNCTIONAL INGREDIENT TO IMPROVE SURVIVAL OF EUROPEAN SEABASS (*Dicentrarchus labrax*) JUVENILES UNDER THERMAL CHALLENGE

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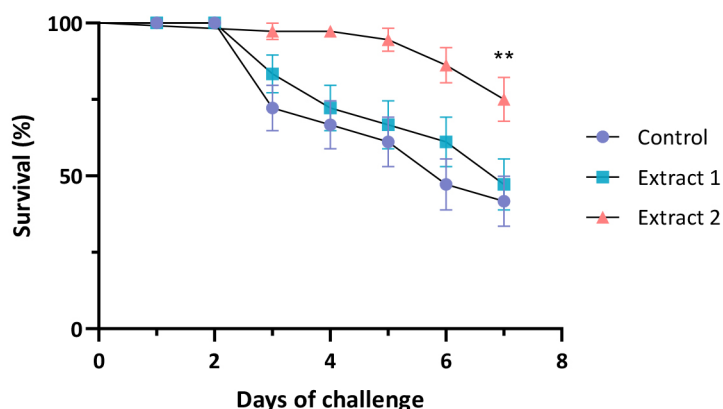
Introduction

The production of agro-industrial by-products has increased in recent years, posing environmental problems and economic challenges regarding their valorization within the circular economy. Over 54 million tons of peanuts (*Arachis hypogaea*) are produced annually, resulting in approximately 0.8 to 1.6 million tons of peanut peels (PP) that are typically discarded despite their potential for valorization as aquafeed ingredients (FAO, 2025). PPs are rich in polyphenols, including flavonoids, procyanidins, and phenolic acids, that exhibit high antioxidant activity. With aquaculture intensification and global warming, fish are increasingly exposed to thermal challenges, leading to increased oxidative stress and disease susceptibility. Dietary supplementation with antioxidants may help maintain redox balance and improve fish resilience to thermal stress. In this context, identifying sustainable and natural antioxidants is of utmost importance to address this gap. This study aimed to: 1) optimize PP extraction to maximize bioactive compounds yield; 2) evaluate the potential of the selected extract on improving zootechnical performance and survival of European seabass under normal and thermal challenge rearing conditions.

Materials and Methods

PP extraction was performed using 50% (v/v) ethanol and water in a microwave-assisted extraction. A full factorial design with solid-liquid ratio (SLR) and extraction time as independent variables was used to determine the optimal conditions to maximize the yield of bioactive compounds from PP. SLR of 1:35, 1:45, and 1:55 (w/v), and extraction times of 30, 60, and 90 s were tested, followed by a 30-minute soaking. Then, all mixtures were vacuum-filtered, and total antioxidant activity, total phenols, ferric reducing antioxidant power (FRAP), flavonoids, and total protein were evaluated. Data from the full factorial design were analyzed using the response surface methodology in Statistica 10. The theoretical optimum (SLR 1:55 for 90s) yielded the highest bioactive compound content. However, due to practical limitations associated with the high solvent volume, the SLR 1:35 and 90s extract was selected for diet inclusion.

Figure 1 – Kaplan-Meier survival curves of European seabass fed the experimental diets during the 7-day thermal challenge. Data is represented as mean (n=3) and standard deviation. Significant differences are denoted by asterisks ** (p-value = 0.002).



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Three isoproteic (46% CP) and isolipidic (18% CL) diets were formulated: a control diet with no extract and two diets containing the selected PP extract (SLR 1:35 and 90s) to enrich the control diet with 500 ppm and 1000 ppm total antioxidants (Diets Extract 1 and 2), respectively. PP extract was mixed with the diets in liquid form, then dried at 40 °C for 48 h to remove water and ethanol. Triplicate groups of European seabass (*Dicentrarchus labrax*) juveniles (IBW: 74 g) were fed these diets to satiation for 4 weeks in a RAS system at 24 °C. After the feeding trial, fish were subjected to a 7-day thermal challenge (temperature increased to 30 °C over 2 days and oxygen maintained at 4 mg/L) to simulate heatwave conditions. At the end of the feeding trial, zootechnical performance parameters were recorded, and during the thermal challenge, mortality was registered. Data on zootechnical performance were analyzed using one-way ANOVA ($p < 0.05$). Survival was analyzed using the Kaplan–Meier method, and group differences were assessed using the log-rank test in GraphPad 8.

Results

Overall, the experimental and predicted values from the response surface model were consistent, supporting the model's robustness. Increasing SLR and time generally improved DPPH, phenols, soluble protein, FRAP, and flavonoids contents, while DPPH decreased with increasing extraction time. The extract SRL 1:55; 90s had the highest levels of phenolics, soluble protein, FRAP, and flavonoids. Nonetheless, due to the limitation of including high solvent volumes in the diets, the SLR 1:35; 90s extract was selected and scaled up for inclusion, as it also provided a high antioxidant yield. The experimental diets (Extracts 1 and 2) did not affect weight gain, feed intake, feed efficiency, DGI, or PER compared to the control diet. During the thermal challenge, survival of fish fed the Extract 2 diet was higher than that of fish fed the control and Extract 1 diets (Figure 1).

Conclusion

PP extracts have high antioxidant content and the potential to increase fish survival under chronic thermal stress when supplemented to diets at 1000 ppm.

Acknowledgments

LV and RM were supported by the Portuguese Foundation for Science and Technology (FCT) through a doctoral grant (2023.02516.BD) and a CEEC Individual contract (DOI <https://doi.org/10.54499/2023.09082.CEECIND/CP2848/CT0017>), respectively. This work was funded by the FCT-funded project “Zero-Waste Omega-3: A Green Label Solution for Sustainable Aquaculture Feeds (DOI: <https://doi.org/10.54499/2023.13017.PEX>).

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GREEN EXTRACTION METHODS IMPROVE PEANUT PEEL ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES: A BOOST FOR SUSTAINABLE AQUACULTURE

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Introduction

Aquaculture intensification has increased welfare challenges, which increase fish oxidative stress and disease susceptibility. Consequently, antioxidant supplementation in aquafeeds is commonly required to maintain oxidative status and enhance fish health. Recently, the European Commission has imposed stricter regulations on the use of synthetic antioxidants such as BHT and BHA and banned ethoxyquin as a feed additive. Moreover, consumer concerns about synthetic antioxidants are increasing, and the search for natural antioxidants is a priority for the aquafeed sector. Agro-industrial by-products have emerged as promising sources of natural antioxidants, offering the added benefit of contributing to circular economy strategies. Peanut peel (PP) is a by-product of the peanut industry with limited applications. It stands out for its antioxidant and antimicrobial properties, mainly attributed to its content of polyphenols and phenolic acids (Dean, 2020). Despite the richness of bioactive compounds, the use of PP extracts as functional ingredients has seldom been evaluated to date.

Hence, this study aimed to optimize the extraction of bioactive compounds from PP using green, low-carbon, and economically viable extraction methods and solvents and to evaluate the antimicrobial activity of extracts with the highest yields of bioactive compounds against pathogenic bacteria.

Materials and Methods

The PP extraction procedure was done with two solvents, water and 50% ethanol (1:1 ethanol/water v/v), using four physical green extraction methods (microwave for 30 seconds, thermal at 70 °C for 30 minutes, ultrasound with probe for 15 minutes, and soaking at 25 °C for 16 hours), followed by a 30-minute soaking with agitation, except for the soaking extraction. All extractions were performed in triplicate in a 1:35 solid/liquid ratio (w/v) and vacuum-filtered. Thereafter, total antioxidant activity, total phenols, flavonoids, and ortho-diphenols were determined.

Ethanol extracts contained higher bioactive compounds than aqueous extracts and were selected to assess PP functional properties. For that purpose, ethanol extracts were evaporated under reduced pressure in a rotary evaporator at 40 °C, freeze-dried for 48 h, resuspended in DMSO, and diluted in PBS to a final concentration of 20 mg/mL.

The antimicrobial capacity of PP ethanol extracts was determined using the broth microdilution assay. Extracts were tested in triplicate against the following marine fish pathogenic bacteria at 1 mg/mL: *Edwardsiella tarda* DSM30052, *Pseudomonas anguilliseptica* DSM1211, *Vibrio anguillarum* ATCC19264, *Lactococcus garvieae* DSM20684, *Yersinia ruckeri* ATCC29473, *Aeromonas hydrophila* DSM3018, *V. harveyi*, and *A. sobria*, the final two isolated from diseased *Dicentrarchus labrax* and *Oncorhynchus mykiss* juveniles, respectively. Enrofloxacin and DMSO were used as positive and negative controls, respectively. The probiotic activity of PP was determined by the potential to improve the growth of probiotic *Bacillus spizizenii* ATCC6633 and *P. chlororaphis* subsp. *piscium* DSM21509. Bacterial growth was measured by optical density at 625 nm (OD₆₂₅) after 24 h and 48 h of incubation.

Differences in PP extraction were determined by a two-way ANOVA with solvents and extraction method as factors. When an interaction was observed, a one-way ANOVA was used for each independent variable. Differences among extraction methods in the antimicrobial assay were assessed using one-way ANOVA, and two-way ANOVA was used to assess differences between the extraction method and growth time in the probiotic bacteria assay. When significant differences were detected (p<0.05), Tukey's multiple-comparison test was used to compare groups. Statistical analyses were performed using SPSS.

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Results

Overall, total antioxidant activity, phenols, flavonoids, and ortho-diphenols were higher when PP was extracted with ethanol than with water. In the ethanolic extracts, antioxidant activity was highest with thermal treatment, flavonoids with ultrasound, and ortho-diphenols with microwave. As ethanol extracts yielded higher bioactive compound levels than aqueous extracts, antimicrobial and probiotic assays were performed with the ethanolic ones. *A. hydrophila* growth was lower when exposed to the extract obtained by microwave extraction compared to other methods. The remaining pathogenic bacterial growth was not affected by the extraction methods. Microwave and ultrasound extracts exhibited the highest antimicrobial activity in absolute values, inhibiting bacterial growth to levels similar to those of the antibiotic used as a positive control, particularly against *E. tarda* and *V. harvey*. The extracts promoted the growth of probiotic bacteria, although no differences were observed among the extraction methods.

Conclusion

Overall, this study showed that PP has high antioxidant and antimicrobial potential for inclusion in aquafeeds. Bioactive compounds from PP were efficiently extracted using 50% ethanol as the solvent, and thermal, microwave, and ultrasound extraction methods yielded the highest extraction. All extracts similarly inhibited pathogenic bacterial growth, with microwave and ultrasound extraction showing the greatest inhibitory effect. All extracts inhibited the growth of *E. tarda* and *V. harvey* to levels similar to the enrofloxacin control. These results highlight the potential of PP inclusion in aquafeeds to improve fish health and microbial resistance.

Acknowledgments

LV, MG, and RM were supported by the Portuguese Foundation for Science and Technology (FCT) through a doctoral grant (2023.02516.BD) and two CEEC Individual contracts (2024.10672.CEECIND and 2023.09082.CEECIND, respectively). This work was funded by the FCT-funded project Zero-Waste Omega-3: A Green Label Solution for Sustainable Aquaculture Feeds (DOI: <https://doi.org/10.54499/2023.13017.PEX>).

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VALORIZATION OF PEANUT PEEL TO BOOST ANTIOXIDANT AND IMMUNE RESPONSES IN GILTHEAD SEABREAM (*Sparus aurata*): AN EX VIVO APPROACH

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Introduction

Global peanut production reached approximately 54 million tons in 2023, and its processing generates an estimated 1.5 million tons of peanut peels per year, most of which are still discarded despite their recognized value as a natural antioxidant source (FAO, 2024). Peanut peel is an agro-industrial by-product with low utilization and is rich in polyphenols, including flavonoids, with well-documented antioxidant activity of interest to the feed industries. However, most of these compounds are physically retained within the peel's lignocellulosic matrix, which limits their extraction and availability. Microwave-assisted extraction (MAE), using either ethanol or natural deep eutectic solvents (NADES), disrupts the peanut peel matrix and enhances the release of bioactive compounds, making the extracts suitable for dietary supplementation. Testing such extracts using an *ex vivo* approach, on intestinal explants rather than live animals, follows the 3Rs principle by reducing animal use and suffering. This study aimed to assess the immunomodulatory and antioxidant effects of peanut peel extracts obtained using ethanol or natural deep eutectic solvents (NADES) in anterior intestinal explants from gilthead seabream (*Sparus aurata*) juveniles, before and after an oxidative challenge.

Materials and Methods

Peanut peel extracts were obtained using 50% ethanol (1:1 ethanol, v/v) and NADES (betaine-urea; 1:2 molar ratio, containing 30% water, heated to 90 °C until homogeneous) as solvents. The extract was obtained by microwave-assisted extraction (MAE) at a solid-to-liquid ratio of 1:35 (w/v) and an extraction power of 700 W for 90 s, followed by a 30 min soaking period. The resulting extracts were centrifuged, and the supernatants were collected and stored at –20 °C. Ethanol was subsequently removed from the ethanolic extract using a rotary evaporator at 40 °C.

A preliminary screening assay was performed to evaluate the cytotoxicity of both extracts using concentrations of 1, 2, 5, 8, and 10%. For this purpose, anterior intestinal explants from gilthead seabream juveniles were collected and subjected to the MTT assay (Erickson-DiRenzo et al., 2015). Explants treated with 50% DMSO served as the non-viable control, and cell viability was determined by measuring formazan absorbance at 570 nm. Three biological replicates were used for each treatment (n = 3). Based on the screening results, extract concentrations of 0.5%, 1%, and 2% were selected for the subsequent *ex vivo* experiment.

Individual intestinal explants (n = 6 per treatment) were incubated for 2 h in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 100 U/mL penicillin–streptomycin, 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 5.5 mM glucose, in the presence of the selected concentrations of either ethanolic or NADES extracts, or without extract (control). Following this incubation, pre-challenge samples were individually collected and stored in RNeasy lysis buffer at –80 °C. The remaining explants were then exposed to a 0.04% (v/v) hydrogen peroxide (H₂O₂) challenge for 2 h under the same culture conditions. At the end of the challenge, explants were individually collected, preserved in RNeasy lysis buffer, and stored at –80 °C until RNA extraction and subsequent gene expression analysis.

Gene expression data were analyzed using a two-way ANOVA with inclusion level and solvent as independent factors, followed by another two-way ANOVA with extract and time as independent factors.

Results

The viability assay showed that the ethanolic extract did not affect intestinal explant viability at any tested concentration. In contrast, NADES concentrations above 2% significantly reduced viability to levels comparable to the non-viable control (50% DMSO). Therefore, only the 0.5%, 1%, and 2% extract concentrations were selected for the subsequent *ex vivo* experiment.

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Gene expression was evaluated before and after hydrogen peroxide (H₂O₂) challenge. Before the challenge, explants treated with the ethanolic extract (0.5% and 1%) exhibited higher levels of the apoptosis marker *casp3*, the cytokines *il-8* and *tgf-β*, and the antioxidant gene *gpx* than those treated with the corresponding NADES extracts. Conversely, NADES treated explants showed lower expression of the pro-inflammatory cytokines *il-1β* and *il-6*, as well as the antioxidant genes *cat* and *sod*, relative to the control. Following H₂O₂ challenge, expression levels of *casp3*, *tgf-β*, and *gpx* decreased, whereas *il-8* expression increased across treatments. Compared with the control after the challenge, NADES 2% treatment exhibited higher expression of *il-6*, *gpx*, and the tight junction marker *ocl*, indicating a distinct transcriptional response to oxidative stress.

Conclusion

Overall, peanut peel extracts modulated intestinal gene expression in a solvent-dependent manner. The NADES extract, particularly at 2%, enhanced the expression of *gpx* and *ocl* in response to oxidative stress, highlighting its potential as a sustainable functional ingredient for aquaculture.

Acknowledgments

LV and FF were supported by the Portuguese Foundation for Science and Technology (FCT) through a doctoral grant (2023.02516.BD) and an investigation grant under the InsectAqua project - Transformation of insect by-products to improve the sustainability of aquafeeds (2023.12923.PEX).

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USING NUTRITIONAL MODELLING TO SUPPORT THE INTERPRETATION OF FEEDING TRIALS WITH ALTERNATIVE PROTEIN INGREDIENTS: INSIGHTS FROM THE CIPROMED PROJECT

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Introduction

The evaluation of alternative protein ingredients for aquafeeds relies on feeding trials that are costly, time-consuming and often require further interpretation beyond conventional performance indicators. Nutritional modelling is increasingly recognised as a complementary tool to experimental nutrition, supporting both diet formulation and the interpretation of biological responses. However, its application to novel ingredients remains challenging due to the limited availability and variability of key nutritional parameters, particularly nutrient digestibility.

Within the CIPROMED project, FEEDNETICS™ was integrated into the formulation and follow-up evaluation of experimental diets for rainbow trout (*Oncorhynchus mykiss*) containing black soldier fly (*Hermetia illucens*) meal and *Spirulina*. Conservative assumptions were adopted during formulation using the best available nutritional information, with subsequent validation based on project-generated biological data.

Materials and Methods

Experimental diets were produced by SPAROS and evaluated in rainbow trout feeding trials conducted by the University of Turin (UNITO). Following completion of the trial, FEEDNETICS™ was used to perform a follow-up analysis using the complete dataset, including all dietary treatments and tank replicates.

Predicted growth trajectories were retrospectively compared with observed body weight data. Model performance was evaluated using Mean Absolute Percentage Error (MAPE) to quantify the agreement between predictions and experimental observations.

Results and Discussion

Predicted and observed growth trajectories showed good overall agreement across all the dietary treatments. The MAPE values ranged from 1.4 to 6.2%, with most dietary treatments presenting prediction errors below 5%, providing an initial validation of the modelling approach using project-generated biological data.

Similar growth performance was observed among the dietary treatments, indicating limited biological differentiation under the experimental conditions. Under these circumstances, FEEDNETICS™ proved particularly valuable as a support tool for interpreting trial outcomes, evaluating formulation assumptions and identifying opportunities for future model refinement.

The integration of additional datasets currently being generated within the CIPROMED project for gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) will enable further validation of the modelling framework and support future extrapolation analyses where biologically meaningful differences are identified.

Conclusions

These preliminary results demonstrate that nutritional modelling can effectively complement experimental feeding trials by supporting diet formulation, validating model assumptions and facilitating interpretation of biological outcomes. Rather than replacing experimental work, FEEDNETICS™ provides an additional decision-support framework that enhances understanding of feeding trial results and contributes to the evaluation of alternative protein ingredients for aquaculture.

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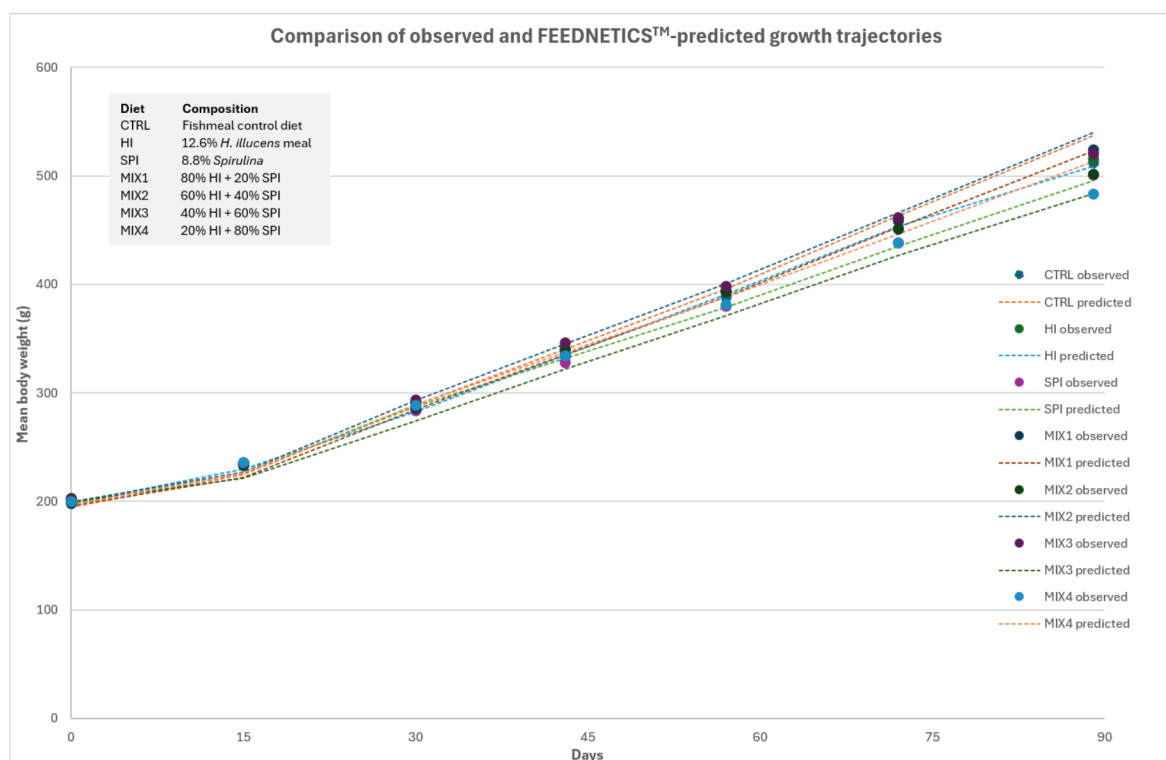


Figure 1. Comparison of observed treatment mean body weight (circles) and FEEDNETICS™-predicted growth trajectories (dashed lines) for rainbow trout fed experimental diets containing different proportions of fishmeal, *Hermetia illucens* meal and *Spirulina*. Treatment-level MAPE values ranged from 1.4% to 6.2%, indicating good agreement between observed and predicted growth trajectories.

Acknowledgment

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Views and opinions expressed are however only the author(s) and that the PRIMA Foundation is not responsible for any use that may be made of the information it contains.

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OFFSHORE FLOATING MULTI-USE-PLATFORMS IN A HIGHLY ENERGETIC UPWELLING REGION

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The availability of wild mussel seed and nutrients are key factors for a successful multi-use scenario in offshore sites. While there is great potential, the transition to high-energy environments introduces challenges, as the hydrodynamic intensity and the consistency of nutrient availability required for commercial feasibility remain insufficiently characterized. Therefore, this study explores the connectivity between the coast of Galicia (Spain), the North of Portugal and the offshore floating wind farms Nor1 (Spain) and Viano do Castelo (Portugal), both in the early planning phase (Fig. 1). The goal is to better understand the role of coastal dynamics in shaping the conditions at the wind farm sites through the export of water masses, nutrients and larvae. The NW Iberian Peninsula is considered as the study site, with its high-energy environment, complex coastal dynamics and established aquaculture sector in the Galician Rías, Spain (Padin et al., 2024).

Introduction

The potential of multi-use wind farms to combine wind energy production and low-trophic aquaculture has been widely explored in the Baltic-North Sea in Europe (Maar et al., 2023). By combining various stakeholders' interests, it is possible to create more sustainable and cost-effective commercial solutions. Current research on the co-location of wind energy and aquaculture is largely restricted to bottom-fixed foundations in semi-exposed waters, leaving the technical and economic feasibility of systems in high-energy offshore environments as a critical area for future investigation (Mascorda Cabre et al., 2021). In addition, the long-term viability of offshore aquaculture depends on consistent larval recruitment and sufficient trophic support to ensure survival and optimal growth rates. Therefore, this study explores the connectivity between coast and offshore floating wind farms using numerical modelling tools.

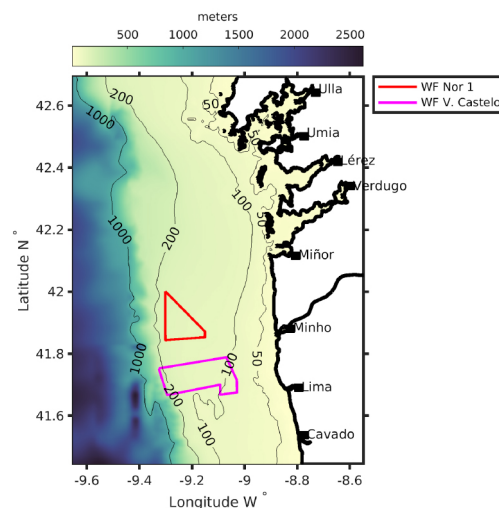


Fig.1: Bathymetry of the study site on a high resolution grid (300 m), demonstrating the wind farm (WF) locations of Nor1 (red), Viana do Castelo (magenta) and main rivers included in this domain.

OFFSHORE FLOATING MULTI-USE-PLATFORMS IN A HIGHLY ENERGETIC UPWELLING REGION

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The availability of wild mussel seed and nutrients are key factors for a successful multi-use scenario in offshore sites. While there is great potential, the transition to high-energy environments introduces challenges, as the hydrodynamic intensity and the consistency of nutrient availability required for commercial feasibility remain insufficiently characterized. Therefore, this study explores the connectivity between the coast of Galicia (Spain), the North of Portugal and the offshore floating wind farms Nor1 (Spain) and Viano do Castelo (Portugal), both in the early planning phase (Fig. 1). The goal is to better understand the role of coastal dynamics in shaping the conditions at the wind farm sites through the export of water masses, nutrients and larvae. The NW Iberian Peninsula is considered as the study site, with its high-energy environment, complex coastal dynamics and established aquaculture sector in the Galician Rías, Spain (Padin et al., 2024).

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Materials and methods

The three-dimensional state-of-the-art Coastal and Regional Ocean COMMunity modelling system (CROCO, v1.3.1) together with the biogeochemical model PISCES were implemented to assess the coastal-offshore connectivity. In addition, the oceanographic model was coupled offline to a Lagrangian Individual Base model Roms OFFline Floats (IBM ROFF) to study the availability of wild mussel seed (*Mytilus galloprovincialis*) in wind farm areas during the peaks of the reproductive cycles. The modelling system uses a two-way nested grid setup. The study area is represented using a high-resolution grid, of approximately 300 m, allowing to simultaneously resolve the oceanic, coastal and estuarine dynamics. These configurations are based on previously well-established modelling systems (Nolasco et al., 2013; Viitak et al., 2026).

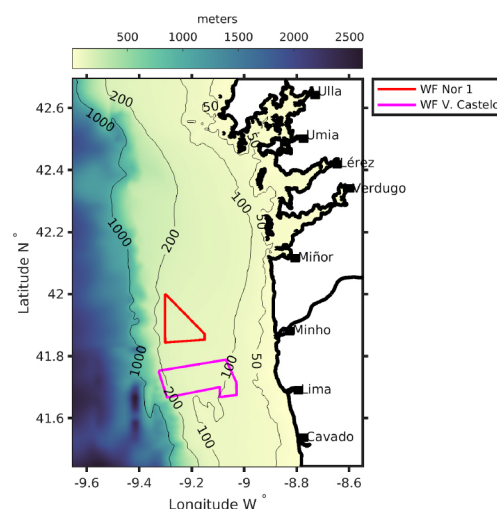


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Results and Discussion

Modelling results indicated that coastal dynamics significantly influence the biogeochemical conditions within the wind farm sites under specific oceanographic regimes. Intense upwelling events promoted the vertical transport of nutrient-rich waters to the surface layers and their offshore export through the surface layers. Galician Rías, as a highly productive region, acted as a source of chlorophyll onto the adjacent ocean, including the wind farm areas. Moreover, during the spring spawning peak of the mussel reproductive cycle, upwelling events promoted the southward and offshore dispersal of larvae. These transport patterns highlight the coast-offshore connectivity, making the wind farm sites as potential settlement habitats for mussel populations.

Acknowledgment

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ENVIRONMENTAL INTERACTIONS OF OFFSHORE CAPTUREBASED ATLANTIC BLUEFIN TUNA AQUACULTURE IN THE BAY OF BISCAY (NE ATLANTIC)

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Introduction

As global fish consumption increases due to population growth, aquaculture has become a critical component of future seafood supply, particularly as yields from capture fisheries stagnate or decline. Aquaculture contributes to food security and economic development but also raises environmental concerns, including organic enrichment, eutrophication, habitat alteration, biodiversity changes, and the potential transmission of pathogens to wild populations.

Material and Methods

Here, we evaluate the potential environmental impacts of a recently deployed offshore Atlantic bluefin tuna (*Thunnus thynnus*) fattening facility located off the Basque coast in the Bay of Biscay. The facility forms part of an innovative pilot project led by the Balfegó Group in collaboration with AZTI, representing one of the first applications of capturebased tuna aquaculture under exposed Northeast Atlantic conditions.

To assess impacts on both the benthic compartment and the water column, we combined environmental DNA (eDNA) metabarcoding, hydrodynamic particletracking modelling, and multivariate statistical analyses. Vertical and horizontal patterns in microbial and microbenthic community structure were compared between reference sites and sites located at increasing proximity to the farming facility.

Compared with traditional biomonitoring approaches based on visual identification of macrobenthic assemblages, eDNAbased techniques offer several advantages, including improved detection of meiofauna and microorganisms, early life stages, and cryptic taxa, as well as greater costeffectiveness and spatial resolution.

Implications

The results of this study evaluate the effectiveness of modern molecular tools for detecting and characterizing environmental interactions associated with offshore tuna aquaculture, building on approaches previously validated for salmon farming in nearshore systems of northern Europe. Ultimately, this work provides novel insights into ecosystem responses to highvalue seafood production systems and contributes to understanding the resilience of offshore marine ecosystems under aquaculture-related pressures.

MACROALGAL GROWTH DYNAMICS IN SEA URCHIN–POLYCHAETE INTEGRATED MULTI-TROPHIC AQUACULTURE

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Introduction

Integrated multi-trophic aquaculture (IMTA) can improve resource-use efficiency by combining species with complementary functional roles. In sea urchin–polychaete systems, polychaetes can recover particulate organic matter, whereas macroalgae may assimilate dissolved inorganic nutrients. However, macroalgal performance remains poorly characterised in coupled recirculating IMTA systems designed to integrate particulate organic matter recovery with dissolved nutrient assimilation. This study evaluated the growth dynamics of *Gracilaria gracilis* and *Ulva lactuca* in a coupled sea urchin–polychaete IMTA system, while assessing dissolved nitrate and phosphate dynamics.

Materials and methods

The experiment was conducted for 4 weeks in three coupled recirculating aquaculture systems (RAS): IMTA–*Gracilaria*, IMTA–*Ulva*, and an IMTA control without macroalgae. Each RAS included sea urchin–polychaete culture tanks connected to a sump, with macroalgae maintained in separate 6 L kreisel tanks at an initial density of 1 g L⁻¹. Macroalgal biomass was recorded at weekly intervals to characterise temporal growth dynamics using specific growth rate (SGR). Water samples were collected twice weekly from each system, with dissolved nitrate and phosphate concentrations quantified by ultraviolet spectrophotometry and the molybdate–ascorbic acid method, respectively. Weekly macroalgal growth was analysed using a mixed-effects model, and associations between growth and nutrient concentrations were assessed using Spearman's rank correlation.

Results

Overall macroalgal SGR did not differ significantly between treatments, averaging 3.65% day⁻¹ for *G. gracilis* and 1.49% day⁻¹ for *U. lactuca*. Weekly macroalgal SGR differed between treatments over time, as indicated by a treatment × week interaction ($p = 0.018$). *G. gracilis* maintained positive growth throughout the trial, peaking at $6.28 \pm 1.16\%$ day⁻¹ in week 2, whereas *U. lactuca* showed rapid initial growth in week 1 ($10.20 \pm 0.56\%$ day⁻¹), followed by declining and negative growth in the final phase. Weekly dissolved nitrate and phosphate concentrations varied between 3.86 and 8.27 mg L⁻¹ NO₃⁻ and between 2.19 and 4.40 mg L⁻¹ PO₄³⁻, respectively, across treatments and weeks. Nitrate declined markedly from week 1 to week 3 in the IMTA–*Ulva* treatment, whereas phosphate remained generally higher in the control. Macroalgal growth was positively associated with nitrate concentration, with a significant relationship observed in *U. lactuca*.

Discussion

Contrasting growth patterns indicated species-specific responses to nutrient availability and system conditions, with *G. gracilis* showing greater stability and *U. lactuca* a transient growth peak followed by decline. The macroalgal SGRs observed were within the broad range reported for *Gracilaria* and *Ulva* spp. cultivated in integrated aquaculture or aquaculture-effluent systems, although the overall SGR of *U. lactuca* was relatively low compared with its initial growth peak. This decline coincided with decreasing nitrate concentrations and thallus bleaching followed by degradation, consistent with previous observations for opportunistic *Ulva* spp., where rapid growth phases have been associated with high nitrate assimilation and subsequent reductions in vegetative stability. In contrast, the sustained positive growth of *G. gracilis* suggests greater temporal stability under the tested conditions, although nitrate and phosphate dynamics alone cannot fully explain this pattern. Growth responses should also be interpreted in relation to culture conditions such as light availability, temperature, hydrodynamics, stocking density, and reactor configuration.

Overall, *G. gracilis* showed greater suitability for sustained performance in this coupled sea urchin–polychaete IMTA system, while maintaining *U. lactuca* as a functional extractive component may require more precise control of biomass and culture conditions.

Acknowledgment

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A STANDARDIZED DIGITAL WORKFLOW FOR AUTOMATED FISH SCALE ANALYSIS IN FISHERIES AND AQUACULTURE

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Fish scale analysis is widely used in fisheries science to assess age, growth, and life-history patterns. However, many existing workflows still rely heavily on manual interpretation and observer-dependent measurements, limiting standardization, reproducibility, and scalability across monitoring programs and experimental studies.

Here, we present a standardized digital workflow for automated fish scale analysis integrating digital sampling, image acquisition, metadata linkage, and quantitative extraction of structural scale features. The workflow combines a custom-built digital measuring system for field and laboratory data collection with automated image-based analysis of scale morphology and circuli patterns.

To evaluate whether scale structures contain biologically meaningful information related to growth conditions, the workflow was applied to an experimental feeding study using tilapia (*Oreochromis* sp.) maintained in experimental recirculating aquaculture systems. During a 12-week grow-out period, two different feeding regimes were applied (7 feeding days per week vs. 5 feeding days per week). In addition, the applicability of the workflow was demonstrated using historical scale material from wild coregonid populations collected over multiple decades.

Fish subjected to continuous feeding developed significantly higher circuli numbers, larger scale dimensions, and altered circuli spacing compared to intermittently fed individuals. Structural differences between feeding groups became increasingly pronounced over the course of the experiment. Application of the workflow to historical coregonid material enabled retrospective reconstruction of age-related growth patterns across multiple decades.

The results demonstrate that fish scales contain quantifiable structural information reflecting growth and feeding history and highlight the potential of standardized digital workflows for scalable fisheries monitoring, aquaculture research, and retrospective ecological analyses. The presented workflow further illustrates the potential of fish scale analysis as a reproducible and scalable tool for documenting fish growth and management history under both experimental and natural conditions.

NOVEL AQUATIC BIODIVERSITY MONITORING ROBOT

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Introduction

Aquaculture systems depend on stable and healthy aquatic ecosystems, yet monitoring water quality and biological communities remains challenging. Conventional monitoring approaches are often time-consuming, costly and largely reactive, making it difficult to detect rapid changes or provide early warnings of deteriorating conditions. Aquatic biodiversity plays an important role in aquaculture ecosystem functioning. Fish ponds with high plankton diversity tend to have better water quality, and show better animal health, and higher yields (Kumar et al., 2020, Lemonnier et al., 2017). Different plankton groups peak at different times, so greater biodiversity provides a more continuous supply of nutritious natural live feed and reduces dependence on artificial feeds.

Our solution

We are developing an autonomous robotic platform that can operate across a wide range of aquatic environments. The system combines advanced environmental sensors, automated biodiversity monitoring and AI-based analysis to provide real-time insight into ecosystem health and enable long-term monitoring of aquatic biodiversity. Off-the-shelf sensors are integrated into the robot.

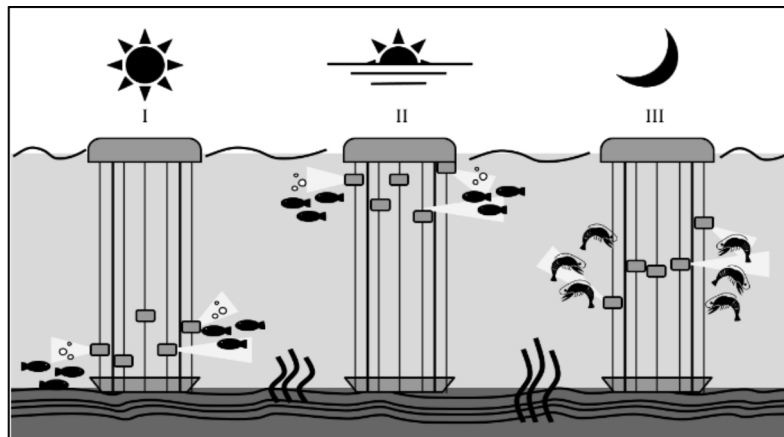


Figure 1

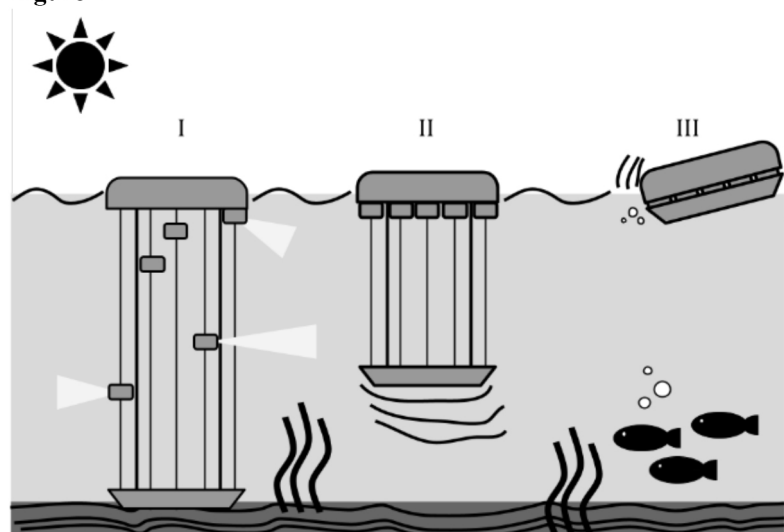


Figure 2

(Continued on next page)

The robotic platform additionally incorporates several novel monitoring approaches. The robot is equipped with a 3D camera for fish detection and identification. A dedicated plankton module uses a specially designed optical system to detect and classify zooplankton. We also integrate “living sensors” in separate modules, using organisms such as *Daphnia* spp. and various bivalves as bioindicators of water quality and pollution.

A key advantage of the system is its ability to monitor both vertically and horizontally. The sensor platform can move up and down the water column to gather measurements at different depths (Figure 1). The entire robot can also relocate autonomously to cover larger areas or multiple sites as needed (Figure 2).

By integrating robotics, AI, automated biodiversity assessment and living sensors, our approach aims to move aquaculture monitoring from periodic, reactive sampling towards continuous and proactive ecosystem surveillance. Such capabilities could support more timely management interventions, improve understanding of ecosystem dynamics and ultimately contribute to more resilient and sustainable aquaculture production.

Acknowledgments

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WASTELESS 2.0: SECONDARY TREATMENT OF RAS WATER AND NUTRIENT REVALORIZATION BY SITE-SPECIFIC MICROBIAL BIOFILM

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To date land based aquaculture systems such as Recirculating Aquaculture Systems (RAS) have to regulate and control the release of excess water (usually rich in Nitrogen (N)). The release of N-rich water in the environment can lead to undesirable eutrophication, and regulations, to avoid and control this, are increasing. Water treatment before release is mandatory and implies a cost to the producer. At present, there is no established system for collection and reuse of RAS release water into new productions. The release from post-smolt RAS is saline and favours production of organisms that tolerate brackish or seawater such as seaweeds or marine microorganisms. In the project WASTELESS we introduce secondary water treatment in RAS by testing the integration of a photo bioreactor (PBR) module. In this module, organisms naturally growing on RAS water will bio-remediate the water and the produced biomass will be investigated for use in fish feed. The area footprint of the reactor treating the effluent from RAS can be minimized by increasing the height and length of the belt, and harvesting of a biofilm is energy efficient compared to harvesting by centrifugation. We aim at establishing multi-species biomass production and use known ecological principles to optimise nutrient removal and cleaning of the RAS water. We have run several experiments mimicking the growth of the natural biofilm present at RAS facility and will present sequencing results indicating the prokaryotic and eukaryotic genera and/or species that dominate the biofilm together with a biochemical profile of the biomass (proteins, lipids). Potentially new ingredients for aquafeed can be produced from the N-rich RAS release by biorefining the produced biomass paste into clean fractions of proteins and fatty acids for example to replace less sustainable ingredients in aquafeed such as soy or fish oil.

SCREENING OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF MACROALGAE COLLECTED FROM THE ADRIATIC SEA

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Introduction

Marine macroalgae are an important source of structurally diverse secondary metabolites with antioxidant and antibacterial properties, making them promising candidates for pharmaceutical, nutraceutical, and food applications. This study evaluated the antioxidant and antibacterial activities of three macroalgal species collected from the Adriatic Sea: the green macroalga *Halimeda tuna*, the red macroalga *Laurencia obtusa*, and the brown macroalga *Ericaria selaginoides*.

Materials and Methods

Macroalgae were collected from the Croatian Adriatic coast, lyophilized, and extracted using an ultrasound-assisted extraction method, followed by fractionation using C18 columns. Methanol (F3) and dichloromethane (F4) fractions were obtained and screened for antioxidant activity using ABTS and DPPH assays. Antibacterial activity was evaluated by the broth microdilution method against three Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis*) and three Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) bacterial strains.

Results and Discussion

Methanol (F3) fractions exhibited markedly higher antioxidant activity than dichloromethane (F4) fractions in both assays. Among the tested species, *E. selaginoides* showed the strongest antioxidant potential, with the lowest IC₅₀ values for ABTS (0.0852 ± 0.0025 mg/mL and 0.6553 ± 0.0237 mg/mL for F3 and F4 fractions, respectively) and DPPH (0.4130 ± 0.0182 mg/mL and 2.1567 ± 0.1041 mg/mL for F3 and F4 fractions, respectively). Macroalga *H. tuna* also demonstrated high antioxidant activity, whereas *L. obtusa* showed comparatively lower activity.

Antibacterial screening revealed consistently greater activity of the methanol fractions in comparison to the dichloromethane fractions. The strongest inhibition was observed against Gram-positive bacteria, particularly *S. aureus* (up to 82.13% inhibition for *L. obtusa* F3 fraction), *S. epidermidis* (94.26% for *H. tuna* F3 fraction), and *B. subtilis* (69.99% for *L. obtusa* F3 fraction). Moderate activity was also detected against Gram-negative bacteria, with *E. coli* showing up to 62.8% inhibition by the *H. tuna* F3 fraction, while *P. aeruginosa* was the least susceptible species.

Conclusions

Adriatic macroalgae possess significant antioxidant and antibacterial activities, particularly their methanolic fractions. The brown macroalga *E. selaginoides* exhibited the highest antioxidant potential, whereas macroalgae *H. tuna* and *L. obtusa* demonstrated the strongest antibacterial effects against Gram-positive bacteria. These findings support the potential of Adriatic macroalgae as a valuable source of natural bioactive compounds for future pharmaceutical and functional food applications.

Acknowledgment

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OTC-MARICULTURE2: AI-BASED PROCESS CONTROL IN AQUACULTURE FACILITIES

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Introduction

Aquaculture plays a vital role in meeting the ever-increasing global demand for seafood, and the industry is currently undergoing a transformation, driven by advances in artificial intelligence (AI) and digitization. In 2022 the production was estimated at 101.4 million tons (FAO, 2025), however, despite the increased demand, the number of aquaculture businesses in Germany have steadily decreased. Between 2011 and 2024, the number of businesses decreased with approximately 58.5 % (Statistisches Bundesamt, 2026). Part of the cause for this decrease lies in the difficult regulation around aquaculture, however, this is not the only reason. Observing the successful businesses, some of their success lies in their ability to monitor their facility, in order to optimize their output. Integrating AI with the Internet of Things (IoT) enables the continuous monitoring of key environmental and operational parameters, such as water quality and fish behavior. This improves operational efficiency, productivity and sustainability, while supporting animal welfare. However, small and medium size businesses can rarely make full use of or afford the large-scale systems available on the market today. In order to bridge this gap, the joint research project “OTC-Mariculture2: AI-based process control in aquaculture facilities”, funded by the German Federal Ministry of Research, Technology and Space (BMFTR), conducted as part of the Ocean Technology Campus Rostock, aims to further develop an autonomous, AI-based monitoring and remote-control system for smaller aquaculture facilities. Furthermore, the project aims to develop methods for additional aquaculture systems and farmed fish species, with the ultimate goal of establishing a universal management system. One of our interim goals is to further improve the recognition model, which was developed during project phase 1, into a universal management system. The intended management system is designed to make the operation of fish farming facilities more efficient. This is made possible by analyzing real-time data from video surveillance and other relevant information (e.g., abiotic factors such as oxygen content, conductivity, and temperature, parasite community and bacteriome). Through the use of a predictive management approach, the measurement, control, and regulation system (MSR system), it will be able to make decisions and recommend specific measures to the facility operator. This enables a targeted response to various events in the monitored facility and contributes to optimizing the success and productivity of the facilities.

AI-based analysis of video data enables adaptive process control, decision support and resource-efficient management, particularly with regard to dynamic feeding strategies. Overall, the project contributes to the development of sustainable aquaculture production systems and aims to support the long-term environmental sustainability of such systems.

Material and Method

Six detection parameters were initially selected for the project. These included the detection of (1) fish eyes, (2) the entire fish, defects in (3) the gill cover, and (4) the entire caudal fin, as well as the (5) upper and (6) lower portions of the caudal fin. To create a recognition model, datasets from mariculture facilities were first annotated based on these six parameters. Additional video data was collected using the camera system to evaluate animal welfare indicators at a rainbow trout mariculture facility in the German Baltic Sea and from the experimental aquaculture system in Born, operated by the State Research Institute for Agriculture and Fisheries of Mecklenburg-Western Pomerania (SRI). Using a recognition model, the collected video surveillance data has been and continues to be statistically analyzed to assess the overall well-being of the fish.

Results

Initial data collection was conducted at the SRI's RAS facility in Born. Underwater video footage of trout during feeding was recorded. The data is generally suitable for the planned AI approaches, although the heavy video compression of the low-cost pond cameras currently in use limits image quality and thus makes it difficult to evaluate fine details. The cameras are sufficient for basic tasks such as fish recognition, but there is room for improvement for more detailed analyses (e.g., minor injuries). First results from the cooperation institutions and statistical evaluation of data from these experiments are presented.

(Continued on next page)

Acknowledgment

The project is funded by the German Federal Ministry of Research, Technology and Space (BMFTR) and conducted as part of the Ocean Technology Campus Rostock. The project consortium consists of the Mecklenburg-Vorpommern Research Centre for Agriculture and Fisheries – Institute of Fisheries (LFA-MV), the Fraunhofer Institute for Computer Graphics Research (IGD), the Professorship for Aquaculture and Sea Ranching at Rostock University, Senect GmbH & Co. KG, and Next Tuna GmbH, a subcontractor of Fraunhofer IGD.

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CRISPR/CAS9-MEDIATED DISRUPTION OF ANP32A REDUCES TILAPIA LAKE VIRUS REPLICATION IN MOZAMBIQUE TILAPIA CELLS

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Tilapia lake virus (TiLV) poses a major challenge to tilapia aquaculture. Identifying host factors that support viral replication can improve understanding of disease susceptibility and inform genetic approaches to disease resistance. This study used CRISPR/Cas9 genome editing to investigate the contribution of acidic nuclear phosphoprotein 32 family member A (ANP32A) to TiLV infection in a Mozambique tilapia brain (OmB) cell line.

An optimised ribonucleoprotein-based CRISPR/Cas9 editing protocol was used to disrupt *anp32a*, followed by single-cell cloning. Sanger sequencing identified a single-nucleotide insertion in exon 3, introducing a frameshift and a premature stop codon. The edited clone and wild-type cells were challenged with TiLV at a multiplicity of infection of 0.01. Viral replication was evaluated by RT-qPCR and infectious virus titration, alongside assessment of cytopathic effects. Transcriptomic and proteomic analyses were integrated to characterise cellular changes associated with *anp32a* disruption under uninfected and infected conditions.

Compared with wild type cells, the *anp32a* disrupted clone exhibited reduced viral RNA abundance and less pronounced cytopathic effects. Infectious virus titres were approximately tenfold lower at 12 days post-infection, indicating reduced, but not abolished, viral replication. Integrated transcriptomic and proteomic analyses revealed changes in pathways associated with mitochondrial function, energy metabolism, aminoacyl-tRNA biosynthesis and protein translation. Concordant reductions in genes and proteins involved in these processes suggest that impaired metabolic and biosynthetic capacity may contribute to the reduced ability of edited cells to support TiLV replication.

These findings support a proviral role for ANP32A in tilapia cells and demonstrate the utility of CRISPR/Cas9 editing for investigating host determinants of TiLV susceptibility. ANP32A represents a candidate for further investigation into genetic approaches to TiLV resistance. Validation in additional independently edited clones and in vivo studies will be needed to establish the reproducibility of the phenotype and assess potential consequences for fish growth and health.

FIRST STEPS TOWARD NEW INDICATORS OF UNCONSCIOUSNESS DURING SHRIMP HARVEST ON FARM?

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Introduction

In terrestrial animals, many indicators of unconsciousness coexist, recognized by the scientific community, some of which are used routinely to limit pain and suffering during slaughter. These indicators may vary depending on the stunning method (captive bolt or electrical stunning). Currently, shrimps *Penaeus vanamei* are primarily slaughtered by thermal shock, although electrical stunning is generating increasing interest. To date, no reliable indicator of unconsciousness has been established in the scientific literature, with the few publications on the subject presenting contradictory results.

Materials and Methods

Development of new indicators and a scoring system

We developed three indicators to assess shrimp reactivity during and after slaughter: eye contact, mouthpart contact, and uropod pinching. Five types of reactions (as opposed to no reaction) to these indicators were recorded:

1. Spontaneous movements present before any contact with the animal;
2. Movements of the locomotor appendages (pleopods and pereopods);
3. Pleopod movements;
4. Pereiopod movements;
5. Attempted escape (jumping).

Tests during harvest in American production ponds

Data were collected in 2023 and 2024, from 746 shrimps reared in Central America, of small size (approximately 9 g) and large size (approximately 20 g). The indicators were assessed in shrimps: (i) immediately after stunning, (ii) after the ice slurry bath (at different temperatures and durations), or (iii) after the combination of both treatments (no data are available for large shrimps that had previously been stunned). Analyses were performed using a binomial model.

Results and discussion

The percentage of reactions varied according to the indicator, shrimp size, and slaughter method. Overall, the proportion of reactions versus no reaction differed from chance (Figure 1). Joint locomotor appendage movements, as well as pereopod movements alone, were more frequent than other reaction types, regardless of shrimp size.

Immediately after stunning, fewer small shrimps reacted when their eyes were touched compared to after an ice slurry bath. No difference in reaction type was observed according to shrimp size.

With prior stunning and after the ice water bath, the number of small shrimps showing a reaction was lower than expected under a random distribution. Without prior stunning, the number of individuals showing a reaction was equivalent (small shrimps) or higher (large shrimps) than expected under a random distribution. The percentage of reactivity was lower in small shrimps when stunning preceded the bath.

Regarding the effect of ice temperature and immersion duration, reactivity decreased for small-sized individuals according to temperature and regardless of the indicator (for large shrimps, this phenomenon was only observed upon mouthpart contact).

Reactivity upon mouthpart contact decreased with immersion duration in ice water at constant temperature, for small shrimps (Figure 2).

Conclusion

The indicators used effectively assess reactivity and are quick and easy to implement during shrimp harvest. The observed reactions raise questions: are they voluntary movements? Indiquent-elles une douleur ?

Many shrimps remain reactive after slaughter, suggesting the need to improve current methods and to take their size into account in slaughter procedures.

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Figure 1. Proportions of reactive and non-reactive individuals, according to the indicators tested, slaughter methods, and individual size, compared to a random proportion (N = 746)

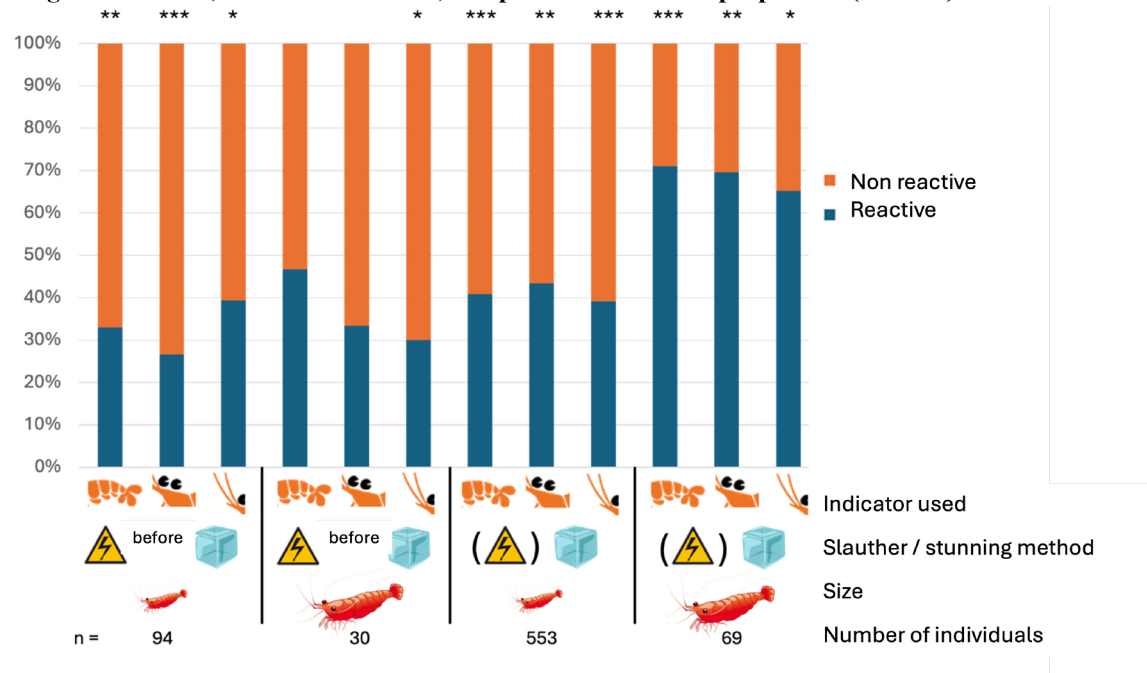
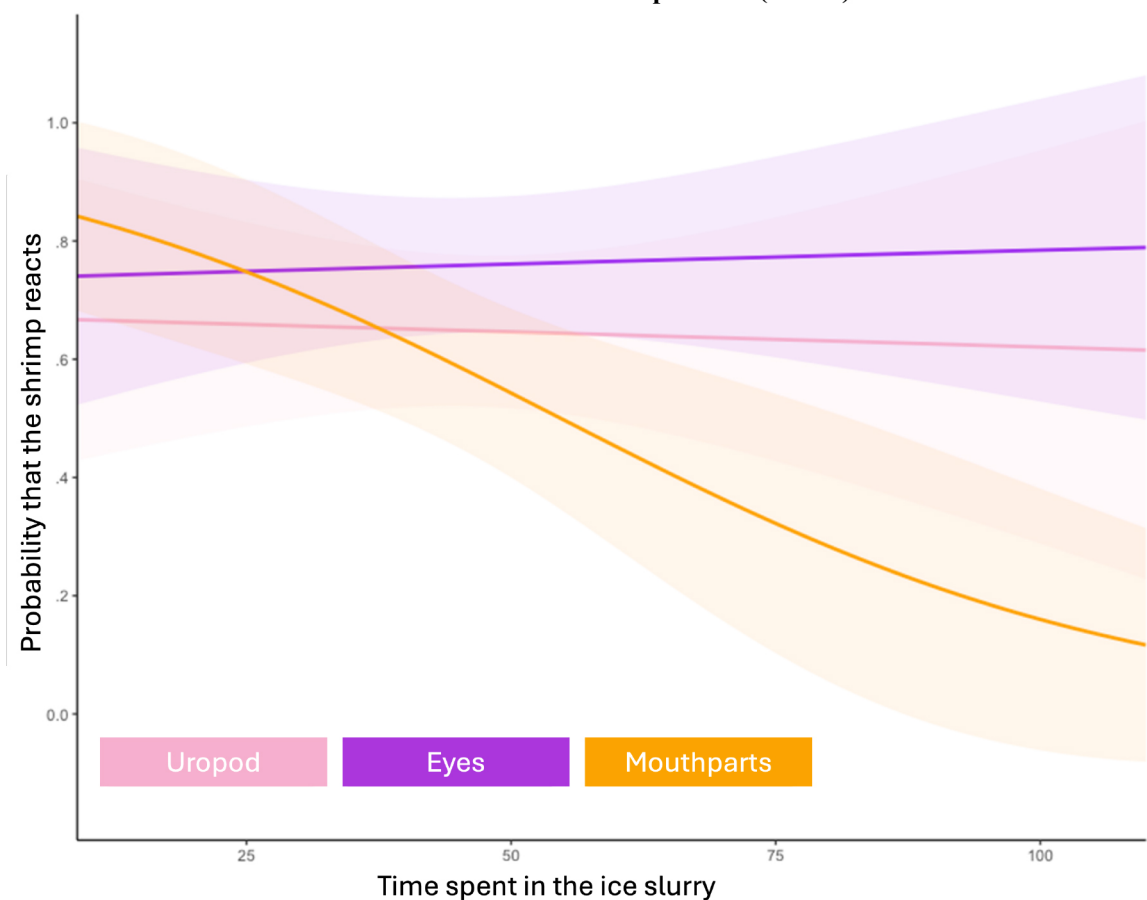


Figure 2. Binomial linear models predicting the reactivity of small-sized shrimps according to immersion duration in the ice water bath at constant temperature (N = 54)



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THE CARE SALMON COLLECTIVE: A NEW COORDINATED RESPONSE TO SOCIETAL EXPECTATIONS

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A unique collective addressing an emerging societal demand: salmon welfare

Consumer concerns regarding salmon are evolving to include animal welfare, a shift supported by robust scientific knowledge coordinated and disseminated through specialized agencies such as EURCAW Aqua (European Reference Centre for Animal Welfare for Aquatic Animals). Various private initiatives focused on fish welfare are emerging, such as the Fish Welfare Initiative, an organization dedicated to the belief that fish welfare is essential for environmental sustainability, business resilience, and global health.

Established in 2024, the CARE Salmon collective brings together key French companies from various sectors, all committed to improving the welfare of farmed salmon. They are supported by an animal welfare NGO (Compassion In World Farming France) and an independent consulting firm (bureau Bankiva), united by a shared goal: to reconcile scientific knowledge, field experience, and operational feasibility, an effort that culminated in the drafting of an open letter.

A quarter of French supplies already aligned with a common baseline

Bringing together ten major players in the French agri-food sector—including wholesalers, processors, and distributors—the collective accounts for over 29% of French salmon imports (50,309 tonnes out of the 200,950 tonnes imported into France in 2025). They all agree on fundamental requirements regarding initial and ongoing professional training, rigorous daily water quality monitoring, the use of health and behavioral indicators to detect signs of stress or disease, fish density management, sea lice control, best practices for handling, transfer, and transport, and humane stunning and slaughter methods, as well as the need to support research and ensure transparency regarding key data for customers and the general public.

The collective welcomes any support: join us at <https://bankiva.fr/en/concertation-animation/collectif-care-salmon/>

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***Penaeus vannamei* BIOFLOC SUPERINTENSIVE CULTURE SYSTEM: STRATEGIES TO REUSE REARING WATERS**

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Introduction

Over the last 20 years, Biofloc Technology Culture System (BFT system) has been developed with the aim of cultivating shrimp with reduced or zero water exchange. Nitrification processes are very well understood. However, the accumulation of certain substances still poses a problem for the BFT system. Nitrate, the final product of the nitrification process, accumulates in the culture water, potentially reaching lethal or sublethal concentrations for farmed shrimp. In addition, phosphate is another substance that can accumulate in the culture tanks. This comes from feeds that contain different forms of phosphate in their formulation. Despite the low toxicity of phosphorus, high concentrations can promote the growth of cyanobacteria. Therefore, experiments have been carried out to (1) reuse culture water, (2) apply denitrification processes to lower nitrate concentrations and release N₂ into the atmosphere, (3) perform phosphate adsorption to extract it from the system and also, (4) use aquatic plants to reduce both nitrate and phosphate concentrations. This paper will present the main results obtained by researchers at the Federal University of Rio Grande (FURG), as a way to mitigate the problems of nitrate and phosphate accumulation in *Penaeus vannamei* crops in BFT systems.

Material and methods

Different experiments were carried out as part of master's dissertations and doctoral theses in the postgraduate program at FURG. In the first phase, tests were conducted to analyze which larger fractions of mature water could be used in a new culture. Subsequently, the minimum percentages (biofloc inoculum) of mature water to accelerate nitrification processes were verified. In the second phase, experiments were carried out to define the best conditions for carrying out denitrification processes in the culture effluents. Subsequently, tests were carried out to perform the denitrification processes simultaneously with the cultures. In the third phase, tests were and are being carried out for the adsorption of phosphate to clays and the reduction of the concentrations of this substance in the culture effluents. In parallel, experiments have been carried out with macroalgae to comparatively analyze the efficiency in removing nitrate and phosphate from shrimp cultures in a BFT system.

Results and discussion

The results obtained in experiments with different water reuse fractions confirmed that it is possible to reuse large quantities, from 75 to 100%, of mature water from a previous cultivation if the nitrate and phosphate concentrations do not exceed safety levels. On the other hand, the minimum concentrations of total solids (TSS) determined for an inoculum to accelerate the nitrification processes of a new cultivation were estimated at 5 mg/L of TSS.

In experiments conducted to define the best conditions for carrying out denitrification processes in cultivation effluents and reuse of this mature water, it was determined that anaerobic denitrification should use organic fertilizers with a C:N ratio of 2 to 4 carbons for each nitrogen in the form of nitrate present in the medium. In tests carried out to analyze denitrification simultaneously with cultivation, it was determined that it is possible to carry out denitrification parallel to cultivation with experimental units equivalent to 25% of the cultivation units. Tests are being conducted by decreasing the size of these units and increasing the frequency of denitrification. In ongoing work to control phosphates, the necessary clay concentrations of 3.5-7.0 g/L were verified over periods of 5 to 10 days. Tests to analyze the comparative efficiency of nitrate and phosphate removal by macroalgae in a BFT system demonstrated the great capacity of these organisms to remove these substances from the water used in shrimp farming in a BFT system. The results confirm that the different methods tested are efficient in improving the water quality of the BFT system, making the use of mature water feasible in shrimp farming under a BFT system. Further work should be carried out to define the best strategies and logistics for reusing mature water over several farming cycles.

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Acknowledgments

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ADVANCES IN HIGH-THROUGHPUT CRISPR SCREENS AND FUNCTIONAL KNOCK-IN'S IN ATLANTIC SALMON

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Introduction

Large-scale aquaculture of Atlantic salmon (*Salmo salar*) is severely impacted by the sea louse *Lepeophtheirus salmonis*, with major consequences for fish welfare and industry sustainability (Godwin et al., 2021). Pacific salmon species exhibit markedly lower lice burdens (Sveen et al., 2025), suggesting intrinsic differences in immune responses during early infection. Understanding the molecular mechanisms that underpin this resistance is a critical step toward a more Pacific-like response in Atlantic salmon. In previous work, we observed multiple genes differentially expressed between species, including macrophage mannose receptor C type 1a (*mrc1a*) (ENSSSAG00000073565), found to be 2.73-fold upregulated in Coho salmon endothelial cells at 36 h post-infection. We posit therefore, that boosting expression of *mrc1a* may enhance immune responses and reduce lice loads in *S. salar*. To test this, we assessed the global transcriptional effects of *mrc1a* modulation using a high-throughput CRISPR-based promoter screen in Atlantic Salmon Kidney (ASK) cells. Following in vitro validation, a 888 bp *hsp8* promoter fragment was introduced upstream of *mrc1a* in fertilized *S. salar* eggs to enable stable overexpression in vivo.

Materials and Methods

pPB TRE dCas9-VPR or pPB TRE dCas9-KRAB constructs were stably transfected into ASK cells to generate CRISPR modulation lines. Cells were transfected with gRNAs targeting regions 50 bp–1 kb upstream of the *mrc1a* coding sequence, followed by RNA extraction and RNA sequencing. For knock ins, fertilized eggs were injected at the singlecell stage with Cas9–gRNA complexes, insertion templates and a HDR enhancer. Embryos were incubated at 8 °C until sampling, when, insertion efficiency was assessed by PCR, and *mrc1a* upregulation was quantified by qPCR.

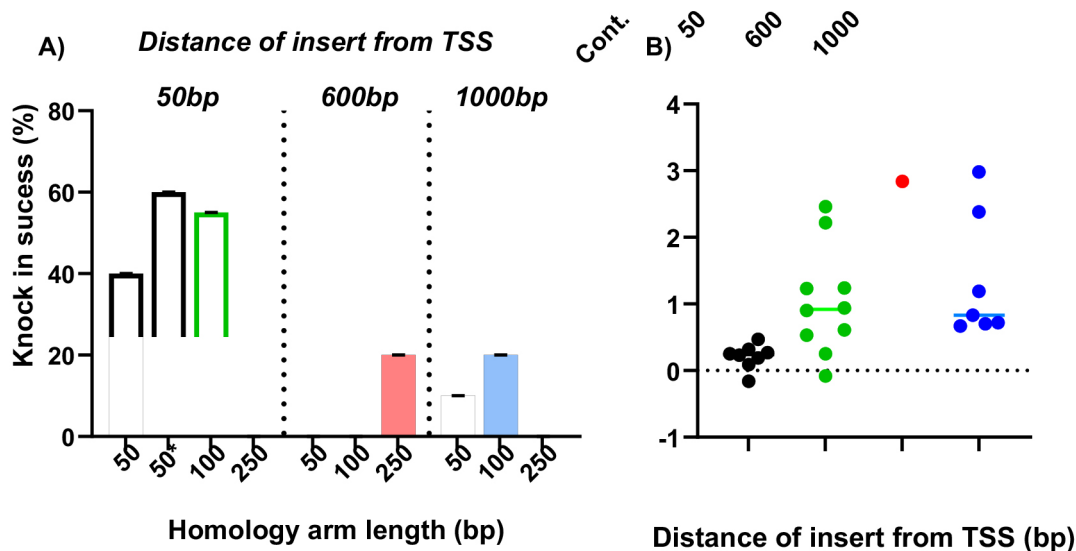


Figure 1, Knock in of hsp8p upstream of mrc1a A) Proportion of embryos that contained successful knock in. Distance of insert upstream of transcription start site (TSS) indicated by partitions. * indicates Enhancer protein was used. Length of each arm homologous to the insertion site of *mrc1a* is indicated on the X axis. Varying the total insert size from 988bp (50bp arms) to 1388bp (250bp arms). B) Expression of *mrc1a*, normalised to housekeeping gene *ef1a*. Each embryo sampled is represented by a data point. Colours in Figure 1B are continuous with the conditions used in Figure 1A.

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Results and Discussion

Stable CRISPR modulation cell lines streamlines experimental workflows, enabling highthroughput screening of large guide libraries relevant to commercially important traits (Yin et al., 2024). This approach is particularly valuable in salmonids, where paralogs may mask phenotypes. Here dCas9-based screening identified genes whose expression was altered following CRISPRa targeting of *mrc1a*. In embryos, no correlation was observed between homology arm length and knock-in success. Which highlights the importance of screening multiple templates for a given target. Use of an HDR enhancer substantially improved large insert efficiency, achieving up to 60%. Although *mrc1a* expression varied due to mosaicism, knock-in groups consistently showed higher expression than controls, with several individuals reaching levels comparable to previous pHSP8-based studies (Reza et al., 2024). Here, we demonstrate the utility of high-throughput dCas systems for screening regulatory effects of industrially relevant genes. This approach provided insights for producing transgenic Atlantic salmon with high-efficiency, functional insertions and can be rapidly applied in the future to gene targets relevant in all fields of aquaculture.

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EVALUATING LANOLIN AS AN ALTERNATIVE FEED INGREDIENT FOR *Litopenaeus vannamei* AQUACULTURE

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Introduction:

Aquaculture production is facing increasing sustainability challenges, particularly in sourcing feed ingredients that can support growth, health and efficient nutrient utilisation while reducing reliance on finite marine resources. There are significant concerns regarding the sustainability of the industry due to the overdependency on fishmeal and fish oil and its environmental impact (Cao et al., 2015). Approximately 26% of global capture fisheries by volume are reduced to fishmeal and fish oil (Majluf et al., 2024). Lanolin is a natural wax derived from sheep wool and is composed mainly of sterols, fatty acids and wax esters (Jenkins and Belsito, 2023). It is currently widely used in pharmaceutical and cosmetic industries due to its emollient properties and complex lipid composition (Sengupta and Behera, 2014). This study provides the first evidence evaluating lanolin in Pacific whiteleg shrimp (*Litopenaeus vannamei*) feeds.

Materials and methods:

Juvenile Pacific whiteleg shrimp (*Litopenaeus vannamei*) were maintained for 12 weeks and assigned to one of four dietary treatments: lanolin-coated, fish oil-coated, soy oil-coated, or uncoated feed. Growth and morphometric measurements were recorded throughout the trial, alongside external welfare assessments based on damage to the body, eyes, antennae, rostrum, and legs. At the end of the feeding period, shrimp were sampled for tissue analysis. Cholesterol concentrations were quantified in hepatopancreas and abdominal tissue to assess dietary effects on cholesterol retention and distribution. Hepatopancreas and midgut samples were collected for histological analysis; and haemolymph and midgut samples were collected for 16S rRNA sequencing (V3-V4 region).

Results:

Dietary treatment significantly influenced growth performance in *Litopenaeus vannamei*. Although shrimp readily consumed the lanolin-coated feed, this treatment resulted in the lowest final body weight, estimated specific growth rate (SGR), and hepatosomatic index (HSI). Apparent feed conversion ratio (aFCR) was also higher in the lanolin diet; however, this was not statistically significant. Shrimp fed the lanolin-coated diet had the lowest final mean body weight (18.21 ± 3.91 g), compared with the uncoated (25.21 ± 3.85 g), fish oil-coated (25.03 ± 4.06 g), and soy oil-coated (23.17 ± 3.79 g) treatments. In contrast, dietary treatment had no significant effect on the overall welfare index. Despite the sterol-rich composition of lanolin, cholesterol concentrations did not differ significantly among dietary treatments in either hepatopancreas or abdominal tissue. Cholesterol concentrations were significantly higher in the hepatopancreas than in abdominal tissue, consistent with its role in lipid metabolism and storage. A significant diet $\times$ tissue interaction indicated that the difference in cholesterol concentration between tissues varied among dietary treatments. Histological and microbiome analyses are ongoing.

Discussion:

Overall, lanolin did not improve growth performance in *L. vannamei* in its raw form. It was hypothesised that lanolin may support comparable or enhanced growth performance due to the crucial role sterols play in crustacean physiology, and their reliance on dietary sources of sterols (Chen et al., 2023; Su et al., 2023; Teshima and Kanazawa, 1971). Previous studies have shown that dietary sterol supplementation can improve growth parameters in juvenile *L. vannamei* (Chen et al., 2023; Guo et al., 2020; Zhang et al., 2019). Despite its sterol-rich composition, lanolin may not provide a readily utilisable sterol source in its raw form. Further refinement, potentially including removal or reduction of specific sterol fractions, may therefore be necessary before lanolin can be considered a suitable lipid ingredient for shrimp feeds.



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INACTIVATION OF SALMON GILL POXVIRUS (SGPV) USING UV IRRADIATION AND CHLORINATION: VIRUCIDAL EFFECT IN ATLANTIC SALMON YOLK SAC LARVAE

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Introduction

Viral pathogens remain a leading cause of mortality in aquaculture industry, posing major challenges to fish health management and biosecurity. Among these, Salmon gill poxvirus (SGPV) represents a significant threat to the global Atlantic salmon aquaculture industry. SGPV, the causative agent of salmon gill poxvirus disease (SGPVD), can cause severe disease characterized by hyperplasia and apoptosis, with respiratory distress and lethargy being typical clinical signs (1). SGPVD outbreaks have been reported in Norway, Scotland and the Faroe Islands (2-5), leading to high mortality rates during the acute phase of infection (3). In Norway, SGPVD have a great economic impact on the salmon farming industry. Unlike other fish viruses, which has been well studied thanks to the development of cell cultures, SGPV cannot be cultured and vaccination is not yet feasible at the early life stages of fish, when these diseases may cause high mortality. Avoiding contact between the fish and the pathogens may thus be the only available control measure. However, methods of inactivation and disinfection of the virus are not available. Here, we investigated potential effect of UV and chlorine on the inactivation of viable SGPV by conducting standardized challenge experimental infection in Atlantic salmon yolk sac larvae.

Material and Method

SGPV isolated from PCR positive Atlantic salmon gill tissue were treated with 10, 25, 90, 250 UVD (mJ/cm²) and 35 mg/L, 60 mg/L, 80 mg/L chlorine concentration. Each UV or chlorine treated SGPV was used to bath-challenge 100 salmon yolk sacs for 7 hr at 6°C in a total volume of 500 mL. After 7 hours, salmon yolk sacs were infected with each UVD (10, 25, 90, 250 mJ/cm²) and chlorine concentration (10 mg/L, 25 mg/L, 35 mg/L, 60 mg/L) were transferred to 5 L tanks, providing separate yolk sac groups. After transfer, the yolk sac was monitored for signs of distress for one hour. The challenge experiment lasted for 30 days, with daily recording of yolk sac mortality in all tanks. Ten yolk sacs were sampled from each treated and the uninfected control group at 7, 14, 21 and 30 DPC, in addition to ten yolk sacs sampled before challenge (0-samples). Fish were anesthetized by immersion in benzocaine (40 mg/L), yolk sac head samples were aseptically collected in RNA-Later (Qiagen, Hilden, Germany) for qPCR, and whole yolk sac were fixed in 10% phosphate buffered formalin for histopathology and immunohistochemistry (IHC). Four methods, RT-PCR, histopathology, immunohistochemistry and in situ hybridization were compared in order to determine the viability of SGPV following inactivation with different doses of UV and chlorine.

Results

All yolk sacs samples (at 7, 14, 21 and 30 DPC) from each UV Irradiation and chlorination treated group, were all negative by PCR. Salmon yolk sacs samples collected from the positive control group at 7, 14, 21 and 30 DPC were positive by PCR. However, there were pronounced differences in the percentage of positive individuals. Results from histology, immunohistochemistry and in situ hybridization, are currently being analyzed and will be updated.

Conclusion

A UV dose of 25 mJ/cm², which is the standard requirement for inlet water treatment in aquaculture facilities, was sufficient to efficiently inactivate poxvirus, comparable to the inactivation observed for VHSV, IHNV, and ISAV. For chlorinated water treatment, the current requirement—an initial residual chlorine concentration of ≥ 50 mg/L and a residual concentration of ≥ 2 mg/L after 25 minutes of treatment was sufficient to inactivate poxvirus.

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STRUCTURAL CARDIAC PLASTICITY IN ATLANTIC BLUEFIN TUNA (*Thunnus thynnus*) UNDER AQUACULTURE AND THERMAL REGIMES

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Introduction

Cardiac responses to aquaculture conditions remain understudied in Atlantic bluefin tuna (ABFT, *Thunnus thynnus*). In other teleosts, such as Atlantic salmon, aquaculture-related stressors can induce morphological deviations and cardiac remodeling (Poppe et al., 2003; Gamperl & Farrell, 2004; Johansen et al., 2011; Frisk et al., 2020; Engdal et al., 2024; Vindas et al., 2024; Hvas et al., 2025). Here, we investigate whether farmed ABFT hearts exhibit morphological and histological differences compared to wild individuals and explore whether these are modulated by temperature.

Materials and Methods

We analyzed 209 hearts from wild and farmed ABFT (juveniles and adults) collected between 2020 and 2024. To evaluate thermal impacts, farmed juveniles were reared at two temperature regimes (mean $\pm$ sd 21.1 ± 4.3 °C, 24.2 ± 3.4 °C). Cardiac morphology was quantified using image analysis (Fiji/ImageJ), focusing on eight shape parameters. Histological sections (HES staining) were used to examine ventricular tissue composition.

Results

Preliminary results show distinct morphological differences between wild and farmed ABFT. Culture conditions are linked to ventricular remodeling in both adults and juveniles, while additionally altering bulbus alignment in adults and increasing ventricular elongation in juveniles. Relative ventricular mass (RVM) is consistently lower in farmed tuna across life stages. Juveniles cultured at higher temperatures exhibit ventricular remodeling and decreased bulbus arteriosus width. Furthermore, variations in ventricular elongation and RVM occur among wild juveniles captured in different geographic areas. Histological analysis reveals altered tissue composition in farmed individuals, more precisely a decreased relative compact myocardium area at both juvenile and adult stages. Final results will detail quantitative differences.

Discussion

This study presents the first quantitative assessment of the ABFT heart, demonstrating morphological and histological differences between farmed and wild ABFT that already emerge in juvenile stages. Linking cardiac responses to environmental conditions such as temperature may help explain how environmental variability influences cardiac performance in ABFT, providing a physiological basis for assessing resilience and adaptation in the wild.

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DOMESTICATION INDUCED CHANGES IN VISION OF EUROPEAN PERCH (*Perca fluviatilis*)

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Introduction

Environmental conditions are a crucial part of shaping the development and organization of the visual system in fish. In aquatic environments light variability, physicochemical properties of water and habitat complexity are only a few factors that directly influence visual performance, while they may also include structural adaptations within the visual system (Fogg et al., 2022; Garcia et al., 2025). The retina, as the primary site of photoreception and initial signal processing, exhibits considerable plasticity in response to environmental conditions. In natural habitats fish are exposed to multifaceted and dynamic visual stimuli, which stimulate complex development of retinal structure (Torres-Dowdall et al., 2017). In contrast, artificial systems, such as recirculating aquaculture systems (RAS), provide relatively simplified and controlled environments with reduced sensory input (Ahmed & Turchini, 2021). These differences in the fundamental characteristics of the environment may influence the development and organization of the visual system in farmed fish species. European perch (*Perca fluviatilis*) is a visually oriented predator which in latest years has display increasing importance in aquaculture. While the majority of its biology is well studied, the effects of domestication on perch vision are poorly understood. Considering short history of domestication and recent technological breakthroughs in production systems of this species (Fontaine & Teletchea, 2019), European perch represents a suitable model for studying environmentally-driven visual plasticity. Therefore, this study aimed to compare the retinal structure and optic tectum organization of wild and domesticated perch to assess the influence of rearing conditions on the visual system.

Materials and Methods

European perch individuals were collected from two environments: a domesticated population reared in RAS (n = 25) and a wild population from pond conditions (n = 25). Morphometric measurements included standard length, total length, body mass, eyeball diameter and pupil diameters. Whole eyeballs were dissected and fixed (Davidson's solution) for histological analysis using standard paraffin techniques and staining protocols (HE and RGB). Retinal morphometry was conducted on microphotographs (n = 36), with retinal layer thickness expressed as a percentage of total retinal thickness. Statistical analysis included one-way ANOVA with significance set at $p < 0.05$.

Results

Morphometric measurements proved that domesticated fish exhibited significantly lower standardized eye diameter along with higher standardized shortest pupil diameter compared to wild individuals. Measurements of retinal layer thickness revealed distinct differences in retinal organization. Wild fish showed greater proportional thickness of inner retinal layers (ganglion cell layer, inner plexiform layer and inner nuclear layer), while domesticated fish exhibited higher proportions of outer layers (outer plexiform layer and outer nuclear layer). Additionally, domesticated fish showed greater proportional thickness of photoreceptor layer (both inner and outer segments). No significant differences were noted in the thickness of the nerve fibre layer between analyzed populations.

Discussion

The observed differences indicate that environmental conditions strongly influence the structural organization of the visual system in European perch. Reduced eye size in domesticated fish may reflect decreased reliance on high-resolution vision under simplified and predictable conditions, while increased pupil size may compensate for reduced light capture efficiency (Papamichail et al., 2025). Enhanced development of inner retinal layers in wild fish suggests greater capacity for signal integration and processing, consistent with the demands of complex natural environments. In contrast, the increased contribution of photoreceptor layers in domesticated fish indicates a shift toward primary signal reception and modulation under reduced sensory input (Fogg et al., 2022).

These findings demonstrate that domestication drives functional reorganization of the eyes of European perch.

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BOOSTING SHRIMP IMMUNITY WITH A CIRCULAR FUNGAL BETA-GLUCAN

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Introduction

One of the most devastating pathogens in shrimp farming is *Vibrio parahaemolyticus*, a major causative agent of White Feces Syndrome (WFS). The reliance on antibiotics for disease control in aquaculture has raised concerns about antimicrobial resistance, environmental contamination, and consumer safety. Consequently, there is growing interest in alternative strategies to enhance the innate immune system of cultured shrimp (Pooljun, C. et al., 2025). Immunostimulants, particularly β -glucans derived from fungal sources, are widely recognised for improving disease resistance in aquatic animals (Uengwetwanit, T. et al., 2025).

This study evaluates the effects of dietary supplementation with *Aspergillus niger*-derived β -glucan (MycoFence®, Citribel) on growth performance, immune parameters, and disease resistance in Pacific white shrimp, *Litopenaeus vannamei*. The effect of dietary supplementation with MycoFence® at three incorporation levels (0.1%, 0.15%, 0.2%) was compared to a diet with incorporation of 2 commercial yeast benchmarks and to a control diet on: (1) growth performance and survival during a 30-day feeding trial; (2) key immune parameters including total hemocyte count, phagocytosis, phenoloxidase activity, superoxide dismutase activity; (3) hepatopancreatic bacterial load; and (4) resistance to experimental challenges with *V. parahaemolyticus* causing WFS.

Figure 1

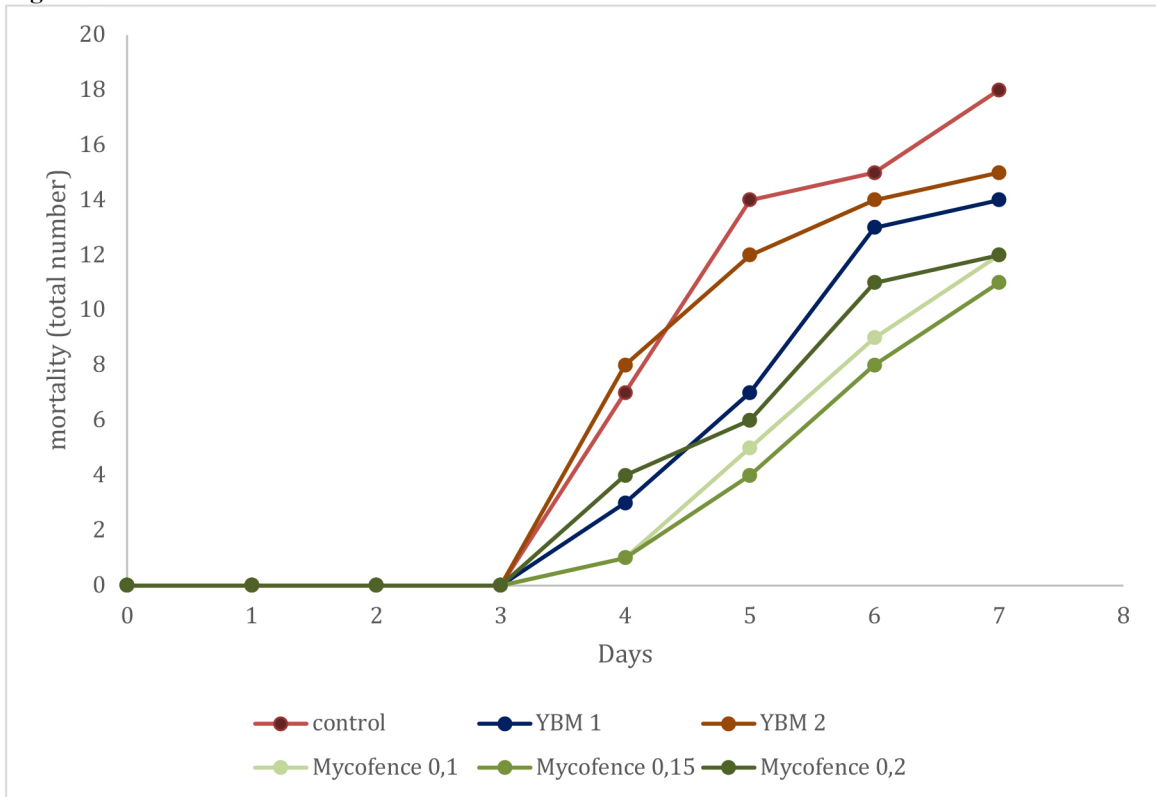


Figure 1. Survival rate (%) of Pacific white shrimp (*Litopenaeus vannamei*) fed diets supplemented with different inclusion levels of MycoFence® as compared to 2 different yeast bench marks (YBM) at 7 Days of WFS challenge. Data are presented as mean (n = 3).

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Results

1500 Pacific white shrimp were fed with 6 different diets for 30 days before and 1 week after the *Vibrio* challenge. Different diet groups were monitored for mortality and samples were taken for immune response parameters after 30 days of feeding. Mortality after the WFS challenge was reduced by the 0,15% and 0,2% MycoFence® incorporation and not by the yeast beta glucan (Figure 1). Mortality in the control group began on Day 2 and continued throughout the observation period, resulting in a final survival rate of 40,00%. All MycoFence®-supplemented groups demonstrated improved survival compared to the control, with clear dose-dependent effects (Figure 1).

Growth performance, hematology markers and absolute quantification of bacteria in different groups will be discussed. These findings demonstrate the potential of *A. niger* β -glucan as a highly effective immunostimulant for shrimp and highlight non-yeast β -glucans as a promising alternative to conventional sources. The superior performance reinforces the potential of *A. niger* β -glucan as a sustainable and environmentally friendly strategy to enhance disease resistance and reduce disease outbreaks in shrimp aquaculture.

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SELENIUM NUTRITION IN ATLANTIC SALMON DURING SMOLTIFICATION IS SYSTEM DRIVEN AND HAS LONG-TERM WELFARE IMPLICATIONS

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Introduction

Interest in selenium (Se) nutrition for Atlantic salmon has grown as diets have shifted from Se-rich fish meal to alternative ingredients that are lower in Se and differ in Se speciation (Aas et al., 2022). Smoltification describes the period during which Atlantic salmon adapt for their transition to sea, with risk of high mortalities in commercial production during the early seawater phase (Björnsson et al., 2011). The Se requirements of Atlantic salmon during this sensitive period remain unknown. Furthermore, rearing conditions may affect requirements, with an increasing use of recirculating aquaculture systems (RAS) in Norwegian smolt production (Kolarevic et al., 2014). Current growth-based recommendations for dietary Se may underestimate the Se needed for tissue homeostasis and welfare (Philip et al., 2025). Therefore, this study aimed to establish Se requirements in Atlantic salmon during smoltification, investigate its long-term effect and their interaction with the rearing environment.

Material and method

A regression design was used to evaluate the effects of graded dietary Se on Atlantic salmon smolt reared in RAS. Seven experimental diets containing graded Se levels (0.45–2.80 mg/kg, Se-yeast) were fed to duplicate tanks of 120 parr (initial BW 37.1 ± 0.5 g). The lowest and highest Se treatments were run in triplicate in both RAS and flowthrough (FT) systems, providing a factorial comparison of rearing environment. After 11 weeks in freshwater, 16 fish per tank were sampled for target tissues and whole fish. Fish from selected treatments were then transferred to sea and fed a common commercial diet (mean Se 0.79 mg/kg) to slaughter size to assess longterm effects of smoltstage Se nutrition. Regression models were fitted to growth data and tissue Se concentrations to estimate optimal dietary Se, and twoway ANOVA was used to test effects of rearing environment.

Results

Smolt reared in RAS showed a slight linear increase in final body weight with higher dietary selenium levels ($p=0.01$, $R^2 < 0.01$, lme), and across both RAS and FT, fish fed Se2.8 had higher specific growth rate than fish fed Se0.4 (0.61 ± 0.05 vs 0.54 ± 0.06 , $p=0.04$, 2-w aov). The whole-body Se concentrations in RAS-reared smolt increased linearly with dietary Se, and the dietary Se concentration required to maintain initial tissue Se level was 1.23 mg/kg feed (CI 1.15-1.29, $p<0.01$, $R^2 = 0.98$, lme). Body weight differences established at the smolt stage persisted three months after transfer to sea, but initially smaller groups showed higher growth, and no significant differences in body weight were observed after six months at sea (3978 ± 1684 g; $p=0.29$, lme). Cataract prevalence at slaughter was significantly reduced with increasing dietary Se levels during smoltification ($p=0.05$, $R^2 = 0.05$, glm).

Discussion

Growth benefits from Se supplementation were observed to range from 0.24 to 2.58 mg/kg across salmonid species (Du et al., 2021; Wang et al., 2018) without a significant effect previously reported in Atlantic salmon (Antony Jesu Prabhu et al., 2020; Lorentzen et al., 1994). In the present study, differences in final body weight were minor and dependent on rearing environment. The reduced cataract prevalence in slaughter size fish indicates a long-term effect of Se supplementation, possibly linked to antioxidant effects in the eye tissue (Bjerkås and Sveier, 2004; Philip et al., 2023). The dietary Se level of 1.23 mg/kg required to maintain whole-body Se homeostasis was above the previously suggested requirement level (0.6-0.9 mg/kg) in Atlantic salmon parr and post-smolt stages (Philip et al., 2025).

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Acknowledgment

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PROGRESS IN X-RAY-BASED DIAGNOSIS OF SKELETAL MALFORMATIONS IN FRAMED FISH

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Introduction: X-rays, methode of choice to diagnose skeletal malformations

For an informed analysis of vertebral column malformations in farmed fish, x-rays remain the method of choice. Today, the skeleton can be also analyzed by micro-ct and nano-ct techniques (Kague et al. 2024), but only x-rays facilitate the examination of large numbers of individuals.

New insights from life x-rays

Initially used to monitor animals after grading or at harvest, technology now also promotes life x-rays. In combination with electronic pit tagging, repetitive x-rays from the same animals let to a new understanding of several type of vertebral column malformations. From repetitive life x-rays we have learned to distinguish malformations that will increase, from those that will not further progress, and from those that can recover. Indeed, not all diagnosed malformations must have a bad prognosis (Witten et al. 2006; Drabikova et al. 2022). Further, we obtain a better understanding about the causes of vertebral column malformations by combining single high quality X-rays with histopathology and other analytical procedures. The combination of analytical procedures helps to determine the severity of malformations and to determine if bone or cartilage is affected. Also, specific types of bone alterations, visible on x-rays, provide information about the health of intervertebral disks. Eventually, for several types of malformations an improved diagnose is possible based on single X-rays only (Fig. 1).

Repair, containment and aggravation

In Atlantic salmon, hyperdense vertebrae (hyper radiodense) are a condition that can be reverted during the production cycle. The same applies to other phosphorous deficiency related bone malformations, detected on x-rays (Fig. 1). Fused vertebral bodies can be remodeled into one single, functional vertebral body. A condition without problems for animal welfare or product quality (Drabikova et al. 2022; Witten et al. 2006, 2019). In the zebrafish model lordosis can be reverted (Printzi et al. 2022). On the other side, specific alterations of intervertebral disks, ribs, haemal and neural foreshadow severe malformations and some malformations only surface late in the production cycle.

Better x-ray quality equals more data

A key for x-ray based diagnosis of skeletal malformation is image quality. Standard x-rays are sufficient to analyze harvest size animals but skeletal malformations that surface late in the production cycle may well start earlier in life. To this end, ultra-high-resolution x-technology can be used to diagnose early life stages in high numbers. This makes it possible to determine period within the production cycle at which skeletal malformations initiate. Ultra-high-resolution x-rays also provide information about internal bone structures, the bone microanatomy, important data (Fig.2).

Conclusions

High quality rays, repetitive x-rays from the same individual and the combination of x-rays with histopathology and other analytical techniques have increased the understanding vertebral column malformations. This helps to provide better diagnosis and prognosis based on radiographs.

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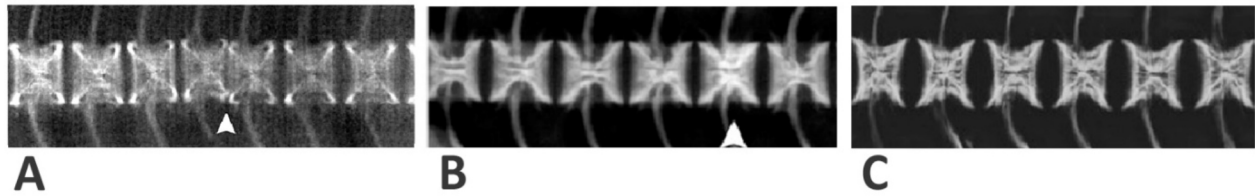
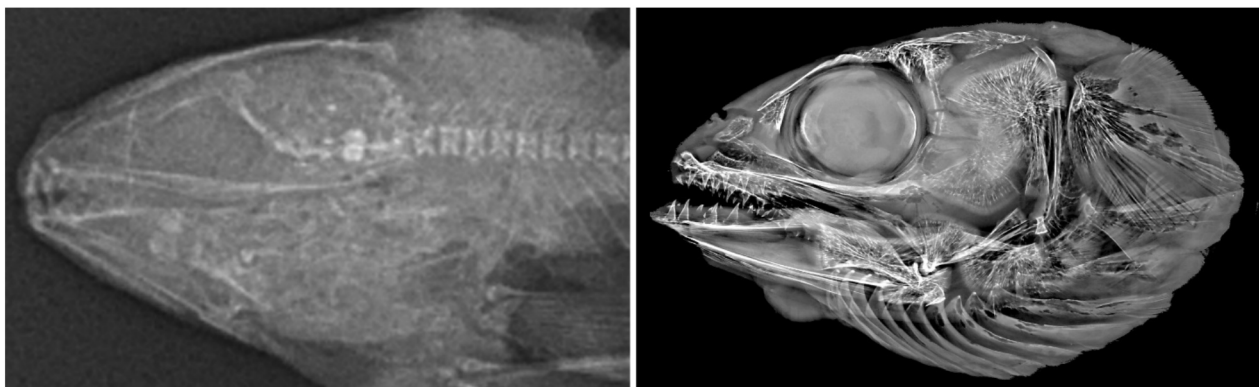


Figure 1. Standard X-rays, Atlantic salmon showing three conditions of phosphorus deficiency. Each condition has a different prognosis. A. Negative prognosis because this condition can develop into a large center of vertebral fusion. B. Hyperdense vertebrae can be completely restored under good husbandry conditions. C. Intervertebral spaces appear too broad. An x-ray artefact related to the presence of large amounts of non-mineralized bone. An immediate increase of dietary phosphorous supply can restore the vertebral bodies.



Head, juvenile salmon: 10 MP standard X-ray tablet

A Faxitron ultra high resolution x-ray shows internal bone structures

Figure 2. High resolution x-rays can be used to diagnose skeletal malformations in juvenile animals, early life stages that are too small for standard x-rays. Also, high resolution x-rays display internal bone structures which is of high diagnostic value.

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SINGLE CELL PROTEIN - THE RIGHT INGREDIENT FOR A SUSTAINABLE AQUACULTURE?

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Introduction

In European aquaculture, protein feeds are essential for the growth and health of farmed fish. Sustainability concerns have led to a shift from fishmeal towards plant-based proteins like soybean meal. Soy production has, however, raised concerns related to pesticide use and deforestation. This has prompted a search for alternative protein sources within Europe. Novel alternatives, such as microbial-derived proteins like Single Cell Protein (SCP), present an innovative solution. In this study, SCP is produced via pyrolysis, converting low-value feedstocks into high-value bioproducts. This study aims to assess the potential environmental and social impacts of this new SCP value chain in aquaculture feed (Figure 1).

Methods

The study employed a “cradle to gate” attributional environmental and social life cycle assessment (respectively E-LCA and S-LCA) approach, considering a SCP production plant located in Norway, following ISO 14044:2006 guidelines for E-LCA and ISO 14075:2024 for S-LCA. SimaPro 9.1 software and the ReCiPe 2016 Midpoint (H) V1.11 method were used for the life cycle impact assessment (LCIA). For the E-LCA, the main database in use was ecoinvent 3.11, while for S-LCA, the social footprint and risks were analyzed using the Social Hotspot Database and its risk map tool, using the Social Hotspots Index (SHI) methodology. In both assessments, expert judgment was applied iteratively to recognize key processes and collect data in critical areas. The selected functional unit was 1 kg of protein, assuming a protein content of 60% in SCP. Mass-based allocation was used when necessary. Considering the maturity of the innovation, a prospective LCA was carried out starting from a screening LCA at an early stage of innovation, using data from laboratory experiments for identifying the major environmental and social “hotspots”, and moving through an intermediate LCA at pilot scale towards a full LCA (data from industrial scale). A literature review using the snowballing methodology identified key features to establish a baseline for scaling up the system. A parameterized model was created to easily adjust contributing inputs as the study progressed from screening to a complete E-LCA. For the industrial scale assessment, the data came from one reference and one optimized scenario, using the SuperPro optimization tool. The model included all the input and output mass fluxes, energy use, and emissions. SuperPro also includes monetary values for materials and utilities, which were used for the S-LCA modelling. The study developed a specific S-LCA framework through several steps. First, social subcategories dependent on the technology were identified, following Hannouf et al. (2024). These were then prioritized using a Responsible Research and Innovation (RRI) approach (Thorstensen & Forsberg, 2016), where stakeholders across two workshops scored the subcategories by perceived importance. A decision matrix was built from these scores, and subcategories rated medium to high importance were included for further scrutiny. Indicators for each subcategory were sourced from existing SLCA frameworks or developed using relevant statistics and expert opinion.

Results and discussion

For the screening LCA, the results show that the SCP final production step is the main contributor to the total climate change emissions, with the other processing steps contributing only to a minor extent. The burdens are mainly linked to energy requirements. According to experts' feedback, however, the energy requirements may be 10-100 times lower under optimized conditions. For the syngas conditioning and fermentation processes, the climate change contribution is mainly associated with chemicals and energy use, while for the sawdust pre-treatment, drying, and pyrolysis steps, it is linked to softwood debarking and processing. Across different SCP protein value chains given in literature, climate change impacts are primarily driven by energy inputs. Bacterial SCP is mainly influenced by natural gas (25%) and electricity use



Figure 1. Single Cell Protein for aquafeed production

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(12%), whereas yeast-based SCP is strongly affected by enzyme production (48.3%) and wheat grain inputs (32%). For the pilot and full-scale E-LCA, the comparison with a generic benchmark can be done by using a prospective data source for energy use simulation of the future energy mix, since energy use has been highlighted as the main driver in the SCP production. For the pilot-scale scenario, shifting the electricity mix from the Netherlands to a Norwegian scenario reduces greenhouse gas emissions (GHG) by 45.8% of the final product. Using a generic market for heat in the future decreased by 46.3% the total GHG emissions related to the base-case scenario. In both models, almost all the indicators present the same trend. The S-LCA results indicate that the main social hotspot is the electricity used for producing the SCP (60% of the total contribution). The social footprint identifies Health and Safety as the main social risk along the supply chain, due to potential occupational risks such as workplace injuries and fatalities, exposure to hazardous substances and unsafe working environment. For the specific assessment, the framework based on the stakeholder engagement activities and literature prioritizes eight social subcategories: the highest score being Health and Safety and the lowest score being Ethical Treatment of Animals. The latest category has not been included in any previous S-LCA studies in literature, and not in the field of aquaculture. Three key indicators were selected for assessing ethical treatment of animals: Feed Conversion Ratio (FCR), Mortality rate, and Gut health.

Conclusions

The environmental and social LCAs have identified key hotspots of the SCP value chain from an early phase of development to a pilot scale following the maturity of the innovation. The results should be used for improving the design of the innovative processes to minimize environmental and social impact before full-scale operation, as well as to provide recommendations to industrial partners during the implementation phase, aiming towards potential benefits for feed security and resource efficiency. The application of S-LCA including animal welfare performance thresholds, and RRI, is important for raising awareness and involving the relevant partners, and it extends beyond the state of the art by including social sustainability in the development of novel bio-based value chains. The inclusion of ethical treatment of animals as a subcategory represents a meaningful extension of traditional S-LCA frameworks, recognizing that animal welfare is a relevant social concern in bio-based value chains such as single cell protein (SCP) production.

Acknowledgements

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CALCEIN FLUORESCENT SHELL MARKING AS A NON-DESTRUCTIVE METHOD TO QUANTIFY GROWTH IN EARLY JUVENILE *Mercenaria mercenaria*

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Introduction

Reliable growth measurements in early juvenile bivalves remain a methodological challenge, as traditional size measurements often lack precision and destructive sampling prevents longitudinal assessment. This study evaluated calcein, a calcium-binding fluorescent dye, as a non-lethal shell growth marker for early juvenile hard clams (*Mercenaria mercenaria*) under hatchery conditions.

Materials & methods

A rough amount of 5,000 juvenile clams (shell length range: 0.6–1.9 mm) were exposed to calcein at a final concentration of 100 mg L⁻¹ for 48 h in aerated seawater (salinity 27–29 ppt; temperature 23–24 °C). A control group (n≈200 individuals) was maintained under identical conditions in aerated seawater for 48 h without calcein exposure to check efficiency of the staining and assess potential mortality related to the staining procedure.

Following staining, clams were transferred to clean, aerated seawater for a recovery period of 48 h, during which survival was monitored. A full water change was done after exposure. A day later, a 50% water change was performed during recovery to remove residual dye.

After recovery, individuals were reared under controlled feeding regime. Shell growth was assessed after weeks using fluorescence microscopy (excitation 460–490 nm) with an Olympus CKX53 inverted microscope equipped with blue/green fluorescence module (Olympus Corporation, Japan).

Calcein incorporation produced a distinct fluorescent band at the shell margin (Figure 1-2). Growth increments were quantified as the distance between the fluorescent mark and the new shell edge using digital image analysis. The method enabled high-resolution measurement of shell accretion at two timepoints (10 days and 80 days after exposure) allowing discrimination of growth differences not detectable using external shell length measurements alone.

Figures

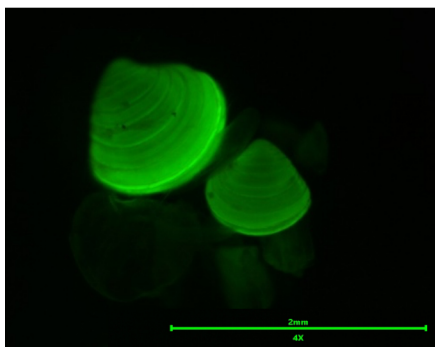


Figure 1: Juvenile *M. mercenaria* 10 days after calcein exposure.

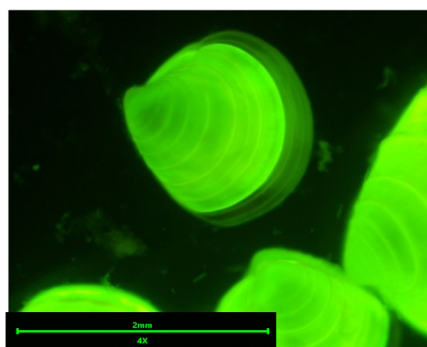


Figure 2: Juvenile *M. mercenaria* 80 days after calcein exposure.

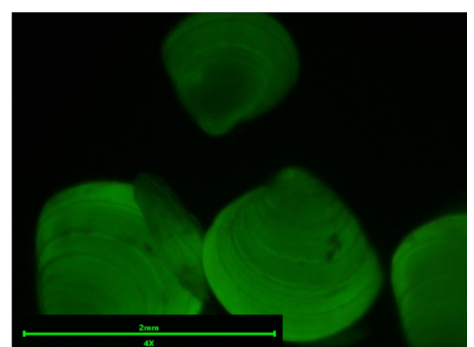


Figure 3: Control juvenile *M. mercenaria* showing natural fluorescence.



Figure 2: Juvenile *M. mercenaria* 80 days after calcein exposure.

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Results and Discussion

Calcein staining produced clear and consistent fluorescent bands in treated individuals, while no fluorescence was detected in control clams. In addition, no mortality was observed during experimental period. Growth increments were measurable across multiple individuals, demonstrating suitability for quantitative assessment under hatchery density conditions.

The technique proved applicable for early juvenile life stages and provided a practical tool for non-destructive growth monitoring. The method supports experimental evaluation of dietary, environmental, and genetic influences on juvenile bivalve growth.

Acknowledgment

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ENHANCING POST-RELEASE SUCCESS IN BALTIC STURGEON *Acipenser oxyrinchus*: EFFECTS OF EARLY-LIFE REARING CONDITIONS ON BEHAVIOR, NEUROPLASTICITY AND MIGRATION

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Introduction

Freshwater ecosystems are biodiversity hotspots but are highly threatened by anthropogenic pressures, leading to rapid species decline [1]. The Baltic sturgeon (*Acipenser oxyrinchus*), once widespread in Northern European rivers and coastal waters, became extinct in the region due to overfishing, pollution, and habitat alterations [2]. Current reintroduction programs rely on hatchery-reared juveniles. However, their success is limited by poor post-release survival. Traditional rearing environments are often homogeneous and may impair behavioral plasticity and adaptability. Environmental enrichment has been proposed to enhance cognitive and behavioral development [3,4]. This study investigated how different early-life rearing conditions affect behavior, neuroplasticity, and post-release performance in Baltic sturgeon.

Materials & Methods

Juvenile sturgeons were reared under three conditions: natural (NAT), semi-natural (CONT), and conventional recirculating aquaculture systems (RAS). Behavioral performance was assessed in a Y-maze (n = 27) by measuring activity, exploration, and latency to novel environments. Post-release movement of NAT and CONT fish (n = 12) was monitored using acoustic telemetry in the River Drawa (Poland). Additionally, brain tissue (n = 27) was analyzed for expression of neuroplasticity markers (BDNF, neuroD, PCNA) using RT-qPCR. Statistical analyses were conducted with significance set at $p < 0.05$.

Results & Discussion

Clear differences in behavioral performance were observed in the different rearing treatments. Fish from RAS conditions exhibited the highest swimming activity, exploration rates, and use of open areas in the maze. While this indicates increased boldness, it likely reflects maladaptive behavior, as excessive activity and risk-prone exploration can increase exposure to predators in natural environments. In contrast, NAT fish showed significantly lower activity, fewer entries into novel compartments, and longer latencies before exploration, suggesting more cautious and energy-efficient behavioral strategies. CONT fish generally displayed intermediate responses.

Performance under flow conditions further emphasized these differences. At the same current velocity NAT and CONT fish maintained position significantly longer than RAS fish, indicating superior swimming endurance and better adaptation to hydraulic challenges. This suggests that exposure to variable flow regimes during rearing enhances physiological performance relevant for riverine environments.

Gene expression analyses supported the behavioral findings. RAS fish showed the lowest expression of neuroplasticity markers (BDNF, neuroD, PCNA), indicating reduced neural development under highly controlled conditions. In contrast, CONT fish exhibited the highest expression levels, while NAT fish showed intermediate values. This pattern suggests that moderate environmental variability—rather than extreme stability—may provide optimal conditions for neural development.

Field telemetry revealed consistent differences in post-release performance. NAT fish initiated movement more rapidly and migrated significantly faster ($10.76 \text{ km day}^{-1}$) than CONT fish (7.46 km day^{-1}), indicating more efficient downstream dispersal. Faster migration likely reduces the duration of vulnerability immediately after release, thereby lowering predation risk and increasing the likelihood of locating suitable habitats. In contrast, CONT fish displayed slower, more variable movement patterns, including temporary residency, which may prolong exposure to threats. Despite these differences in movement dynamics, both groups exhibited similar habitat preferences, primarily occupying mid-channel areas. This suggests that fundamental habitat selection cues are retained across rearing conditions, whereas differences are more pronounced in behavioral flexibility and movement efficiency.

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Overall, the results demonstrate that rearing environment strongly shapes behavioral traits, physiological performance, and neuroplasticity in Baltic sturgeon. Traditional RAS conditions promote high activity but are associated with reduced cognitive flexibility, endurance, and neural development. In contrast, enriched and semi-natural environments enhance cautious behavior, spatial awareness, and migration efficiency—traits that are likely critical for survival in the wild.

These findings highlight the importance of incorporating environmental complexity and moderate variability into hatchery practices for conservation purposes. Optimizing rearing strategies to promote adaptive behavior and neural plasticity may substantially improve post-release survival and contribute to the long-term success of Baltic sturgeon restoration efforts.

Acknowledgement

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CROSSING THE LINE OR CLOSING THE LOOP? COMPARING CONSUMER PERCEPTIONS OF FISH RAISED IN TREATED WASTEWATER-BASED AQUAPONICS IN GERMANY AND ITALY

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Introduction

Global population growth, climate change, and increasing demand for animal-based protein are placing unprecedented pressure on food systems, highlighting the need for more sustainable and resource-efficient production models (Aiking & de Boer, 2020; Webb et al., 2020; Monika Zurek et al., 2022). In this context, aquaculture is expected to play a key role in sustaining future protein supply (Béné et al., 2015; FAO, 2024), yet its environmental sustainability performance varies significantly across production systems (Garlock et al., 2024). Among emerging solutions, aquaponic systems offer a more sustainable approach by integrating fish and plant production while efficiently reusing water and nutrients (Goddek et al., 2019; Horn et al., 2023).

More recently, the integration of treated wastewater (TWW) into such systems has been proposed as an additional step towards more resource-efficient circularity, with potential to further reduce freshwater use and recover valuable nutrients (Cifuentes-Torres et al., 2021). These systems operate within a tightly interconnected human–animal–environment nexus, where water quality, animal welfare, environmental integrity, and food safety are simultaneously intertwined. However, despite its positive environmental potential, this approach remains constrained by regulatory uncertainty, technical concerns, and, most importantly, limited societal acceptance (Christou et al., 2024; WHO, 2004). Whereas the technical aspects of treated wastewater utilization have been extensively investigated, the main research gap lies in the limited exploration of consumer perceptions regarding fish cultivated in treated wastewater based aquaponic systems (Drechsel et al., 2022). To close this gap, this study investigates German and Italian consumers' perceptions of fish produced in treated wastewater-based aquaponic systems.

Methodology

A combination of a systematic literature review and an online panel survey was applied to examine how concern, perceived benefits, and acceptance-enhancing measures shape consumers' willingness to consume fish produced in this system, and how these perceptions relate to the human–animal–environment nexus. The construction of the perception dimensions were informed by the Health Belief Model. The online panel survey targeted only fish consumers through stratified sampling by gender, age, and region of residence both in Germany and Italy, and resulted in 1516 participants.

Exploratory factor analysis using Principal Component Analysis with Varimax rotation was conducted to identify and refine the underlying structure of the perception constructs, with internal consistency assessed prior to extracting factor scores. The dependent variable measured consumers' willingness to consume fish from treated wastewater based aquaponic systems on a six-point scale. After initial attempts with ordinal logistic regression proved unsatisfactory due to category overlap and violations of the proportional odds assumption, most notably in the German sample ($\chi^2(3) = 14.755, p = .002$), multinomial logistic regression was adopted, with perception dimensions used as predictors to assess their influence on consumers' acceptance, uncertainty, or rejection. A Pearson correlation analysis was also conducted to explore associations between perception dimensions and the components of the human, animal, and environmental nexus.

Discussion and Conclusion

Results revealed a general lack of in-depth knowledge of the use of treated wastewater in fish production, with approximately 50% of respondents in both countries (Italy: 52.2%; Germany: 49.7%) having only heard of the concept and not been able to describe it. Exploratory Factor Analysis identified three core perceptual dimensions shaping consumer attitudes: "Concern" (human-centered risk perception), "Perceived Benefits" (environmental and system-level evaluation), and "Acceptance-Enhancing Measures" (trust and governance). Multinomial Logistic Regression analysis showed that "Concern" was the most significant predictor of rejection in both nations.

In Italy, a high level of concern increased the likelihood of belonging to the rejection group by 5.11 times, whereas in Germany, it increased the odds by 3.27 times. Conversely, “Perceived Benefits” served as the primary driver for acceptance, increasing the likelihood of acceptance by 4.92 times in Italy and 3.72 times in Germany. Furthermore, “Acceptance-Enhancing Measures” such as transparency, independent checks, and labeling significantly reduced the likelihood of rejection in Germany (OR = 0.67), though they had a limited effect on active acceptance. Comparative results indicate that Italian consumers are more sensitive to both risk and benefit signals than German consumers, underscoring the need for culturally tailored communication strategies to promote TWW-based aquaculture.

When it comes to the interaction between perception dimensions and the human-animal-environment nexus, Pearson correlation analyses further demonstrated a holistic yet asymmetric relationship between consumer perception dimensions and the nexus. The “Concern” dimension showed a strong, statistically significant negative correlation with all three components in both countries (Italy: $r = -0.682$ to -0.590 ; Germany: $r = -0.626$ to -0.555 ; $p < 0.01$). This indicates that consumer risk perception is not localized but extends across the entire human-animal-environment nexus. Conversely, “Perceived Benefits” were positively and significantly correlated with all nexus components. In addition, Animal health, although integrated into the perception of risk, occupied a secondary position in consumer priorities compared to human and environmental impacts.

Overall, the study demonstrates that the social viability of the fish raised treated wastewater based aquaponic systems highly depends on how consumers cognitively and emotionally interpret the reuse of “human excreta” in food production. By integrating the human-animal-environment nexus into consumer research, this study contributes to a more comprehensive understanding of acceptance dynamics in circular food systems and provides actionable insights for policymakers, aquaculture practitioners, and system developers seeking to foster sustainable food system transitions.

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ALIGNING MOLECULAR AND MICROSCOPY METHODS FOR BIOFOULING DETECTION OF FARMED SEAWEED (*Alaria esculenta* AND *Saccharina latissima*)

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Kelp farming in the North Atlantic is emerging as new sustainable source of food, nutraceuticals and biostimulants. Seaweed biofouling by epibiont taxa, including bryozoans, hydrozoans, amphipods, bivalves, and gastropods is a key constraint in kelp yield and quality. The planktonic stages of these species belonging to meroplankton precedes visible settlement on seaweed fronds. Thus, the precise taxonomic detection of the planktonic stage and the assessment of temporal alignment with the biofouling stage could act as an early warning helping farmers plan harvesting times. To achieve this, monthly samples of plankton and kelp fronds were collected from a Scottish kelp farm during a kelp farming period (September 2021-July 2022). The taxonomic identity of kelp biofouling epibionts was established by microscopy and DNA-barcoding. The taxa were then traced in the plankton samples using both microscopy counts and eDNA-metabarcoding. Taxonomic identification of kelp epibionts via COI-based barcoding agreed with microscopy findings in 14 out of 19 specimens and helped refine the taxonomic resolution from family and genus down to species level. Out of the 20 epibiont species identified from the kelp fronds, 10 were identified via microscopy at phylum, class or genus level and 10 were identified via eDNA metabarcoding at species level. Microscopy counts of hydromedusae, bryozoan cyphonautes and bivalve larvae peaked in the plankton two weeks before the maximum coverage on *Saccharina latissima* fronds. Maxima of eDNA reads and microscopy abundances did not coincide temporally for the same taxa. Depth-stratified frond surveys showed that kelp segments that were deeper in the water column harboured the highest hydrozoan colonisation, indicating either depth-related colonisation processes, kelp age-related exposure effects or UV avoidance. The results indicate that the combination of molecular diagnostics and conventional microscopy improves the sensitivity and specificity of biofouling detection, facilitating earlier and more informed farm management decisions.

POLYCYSTIC LIVER IN FARMED ATLANTIC SALMON (*Salmo salar* L.): GROSS, HISTOLOGICAL AND BIOCHEMICAL CHARACTERIZATION

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Hepatic cysts are rare findings in farmed Atlantic salmon (*Salmo salar* L.) and are typically identified incidentally during routine health examinations. In this study, polycystic liver was identified in farmed post smolt Atlantic salmon during routine health monitoring at Lerang research station (Stavanger, Norway). This study provides gross, histopathological and biochemical characterization of hepatic cysts detected originating from three egg batches. A total of 15 fish were identified with polycystic liver during necropsy examinations conducted in 2023, of which three fish could be used for detailed histological evaluation. Among three fish, cystic fluid and blood plasma from one fish could be secured for biochemical analyses. Affected fish exhibited multiple fluid filled hepatic cysts of varying size and number, occasionally associated with abdominal distension, while other internal organs appeared unaffected. Histopathological examination revealed cyst like structures lacking an epithelial lining and bordered by connective tissue capsules, and were therefore classified as pseudocysts. Adjacent hepatocytes appeared morphologically normal, and no evidence of parasitic, infectious or neoplastic etiology was observed. Biochemical analysis demonstrated that cystic fluid composition closely resembled blood plasma, supporting a transudative origin and a non-infectious, possibly developmental basis for the lesions.

BONE MINERALISATION IN FARMED ATLANTIC SALMON: ROLES OF MINERALS AND VITAMINS

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Optimising mineral nutrition during early life stages is essential for healthy growth and skeletal development in farmed Atlantic salmon (*Salmo salar* L.). Across three freshwater feeding trials, we investigated the roles of dietary phosphorus (P), calcium (Ca), vitamin K, and vitamin D in regulating growth performance, bone mineralisation, and spinal deformities in juvenile Atlantic salmon. Diets were formulated with either recommended P levels or a 20% P reduction, tested with or without Ca supplementation, and combined with varying vitamin K and D inclusion strategies.

Phosphorus inclusion was consistently identified as the principal factor influencing mineral homeostasis and bone mineralization across all studies. A moderate reduction in dietary P combined with elevated Ca intake led to reduced growth, reduced mineral levels, and a comparatively higher incidence of skeletal deformities. Vitamin K levels demonstrated no impact on growth, with hepatic vitamin K remaining stable. Moreover, vitamin K was insufficient to offset reduced phosphorus in promoting bone mineralization, and increases in vitamin D levels did not yield evident benefits for skeletal health.

These results emphasize the need for precise phosphorus nutrition and balanced Ca:P ratios in freshwater startfeeding diets. They also underline the need to avoid unnecessary calcium supplementation, supporting robust growth, bone health, and more sustainable feed formulations.

EFFECTS OF PRODUCTION PROTOCOLS IN RAS AND TRANSFER SIZE ON SEAWATER PERFORMANCE OF ATLANTIC SALMON

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Background

Production of a large salmon smolt is a relatively new strategy in Norwegian salmon farming, and several production protocols are currently used in the land-based phase (Ytrestøyl et al., 2023). Most farmers apply a winter signal with short days (12 hours light and 12 hours dark; 12L:12D) to smoltify the fish, while others apply continuous light (24 h) throughout the production period. Salinity regimes also vary considerably, some facilities use freshwater while others use brackish water. Farmers report variable performance of larger smolts after transfer to sea, but the wide diversity in production strategies combined with year-round sea transfer, makes it difficult to draw conclusions about the factors underlying reduced performance in sea. Thus, documentation is still lacking on which production protocols support growth and survival, while at the same time reduce the risk of early sexual maturation in males (Good and Davidson, 2016).

Through several projects, Nofima and partners have tested and evaluated the effect of salinity (brackish water versus freshwater), photoperiod (continuous light (24 h) versus a winter signal (12L:12D) to induce smoltification) and fish size at sea transfer (120, 320 and 850g) on the seawater performance in Atlantic salmon. Additionally, fish size at the initiation of a winter signal (50 versus 120 g) and winter signal duration (6 weeks and 18 weeks) was examined with respect to growth and performance in seawater cages until slaughter and in seawater tanks for 3 months. In seawater tank trials, the fish were exposed to environmental conditions simulating either spring (6°C and increasing day length) or autumn (12°C and decreasing day length).

Results

The results indicate that exposure to a winter signal protocol generally improved growth in the seawater phase. However, final slaughter weight could still be higher in fish exposed to 24 h light in RAS, largely because they were bigger at the time of sea transfer. Survival and growth in seawater were influenced more strongly by the size and transfer time than photoperiod and/or salinity. Fish transferred later in autumn at a larger size showed poorer growth and higher mortality in the seawater phase than fish transferred at a smaller size earlier in the autumn. However, both season and size could have affected the outcome.

Fish exposed to a winter signal combined with brackish water RAS showed improved growth after transfer to seawater, particularly when transferred at a larger size. However, a winter signal also increased the risk of sexual maturation in males, especially in fresh water. Exposing the fish to low temperature and increasing day length that simulated spring gave less sexual maturation than high temperature and decreasing day length that simulated autumn. Thus, which protocol to use should be selected based on the timing of sea transfer for the larger smolts/post-smolt.

Welfare, quality, seawater tolerance and the incidence of nephrocalcinosis was also assessed in the different production protocols. There were no large effects of photoperiod and salinity in RAS, but slightly higher score for nephrocalcinosis in fish on 24 h light in RAS than in fish given a winter signal. However, smolt development and osmoregulatory capacity, assessed using NKA enzyme activity, in gill, intestine and kidney were differentially affected by photoperiod and salinity protocols. Welfare and quality were not affected by protocol in RAS, but there were some minor effects of experimental conditions in the tanks with seawater, with reduced welfare scores in fish at higher temperatures.

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BEYOND PROTEIN: *Hermetia Illucens*-BASED INGREDIENTS AS FUNCTIONAL PERFORMANCE AND GUT HEALTH DRIVERS FOR FISH

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Introduction

Beyond their value as a source of protein, *Hermetia illucens*-derived ingredients exhibit underexploited functional potential in salmonid nutrition. Their water-soluble fraction, rich in bioactive compounds, such as nucleotides, free amino acids or short-chain peptides are posited to stimulate feed intake and enhance zootechnical performance, while antimicrobial peptides, lauric acid, and chitin could support immunity and gut robustness. Recent industrial advances in black soldier fly larvae (BSFL) production now ensure consistent product quality, enabling reliable expression of these functional effects. This study therefore evaluates BSFL meal as a booster of feed intake, efficiency, and gut health in grower Atlantic salmon (*Salmo salar*).

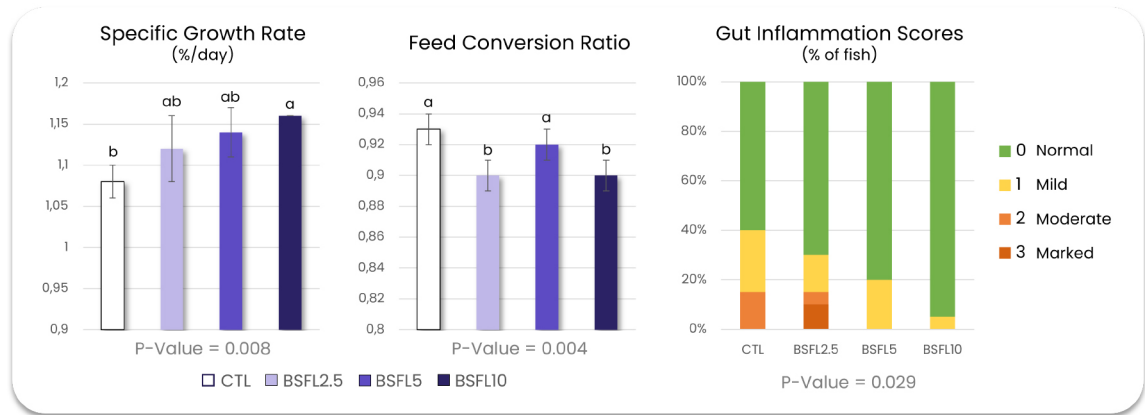
Materials and Methods

In this saltwater trial, adult salmon (~300 g) were fed to apparent satiation three times daily for twelve weeks in randomized quadruplicates. Four diets were tested: a reference diet (CTL) based on fishmeal (18%) and soy protein concentrate (SPC, 25%), and three experimental diets including BSFL meal at respectively 2.5% (BSFL2.5), 5% (BSFL5), and 10% (BSFL10) inclusion rates as an SPC replacement. Daily feed intake as well as initial and final body weights were recorded to calculate standard zootechnical performance indicators (weight gain, WG; specific growth rate, SGR; feed conversion ratio, FCR). Gut samples were also taken for histology analysis: ranking of the gut inflammatory status, villi length, and submucosal thickness.

Results

During this trial, feed intake was found to be increased linearly with dietary BSFL inclusion (423 g/fish for CTL diet to 457 g/fish for BSFL10 diet). Beyond the increased intake, insect meal inclusion also improved feed efficiency, as demonstrated by a significantly higher WG in the 10% BSFL group (+11%, $P = 0.02$) and a reduced FCR in this same group compared to the CTL group (0.90 vs. 0.93; $P = 0.004$). Salmon fed the BSFL2.5 diet demonstrated a significantly improved protein retention efficiency (44.2% for BSFL2.5 vs. 40.3% for CTL diet, $P = 0.001$). Compared to the control, the BSFL-fed groups showed a lower proportion of fish with elevated gut inflammation (Figure 1). Although villus length was not significantly affected by treatment, fish receiving the BSFL meal showed a numerical increase in villus length, which might indicate enhanced nutrient absorption. Concurrently, submucosal thickness was numerically reduced in the BSFL-fed groups compared to the control ($P = 0.099$), providing further evidence of reduced inflammatory effects.

Figure 1: Effects of Dietary Black Soldier Fly Larvae (BSFL) Inclusion on Growth Performance, Feed Efficiency, and Gut Health of adult Atlantic salmon.



(Continued on next page)

Discussion

The results of this study confirm that *Hermetia illucens* meal is a highly functional alternative to traditional protein sources, capable of enhancing both zootechnical performance and intestinal integrity of Atlantic salmon. The physiological basis for these improvements likely stems from the unique bioactive profile of BSFL protein and its positive influence on gut health. The compounds stated previously are recognized for their benefits on intestinal microbiota, natural immunity and stress resilience, and attractivity/palatability. The observed linear increase in feed intake and significant improvements in weight gain and FCR align with previous findings that BSFL meal inclusion can improve growth rates and feed efficiency in juvenile salmon¹, and more broadly fish aquatic species^{2,3}. Our histological observations also support existing research indicating that BSFL meal does not compromise gut health⁴ and positively modulates gut microbiota⁵ of fish.

In conclusion, by demonstrating their functional value in adult Atlantic salmon, these findings confirm that BSFL-derived ingredients can serve as performance drivers beyond their sole nutritional and environmental value.

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PISCINE ORTHOREOVIRUS (PRV-1) AND PISCINE MYOCARDITIS VIRUS (PMCV) INFECTIONS DO NOT IMPAIR THERMAL OXYGEN-USE PERFORMANCE IN ATLANTIC SALMON (*Salmo salar*)

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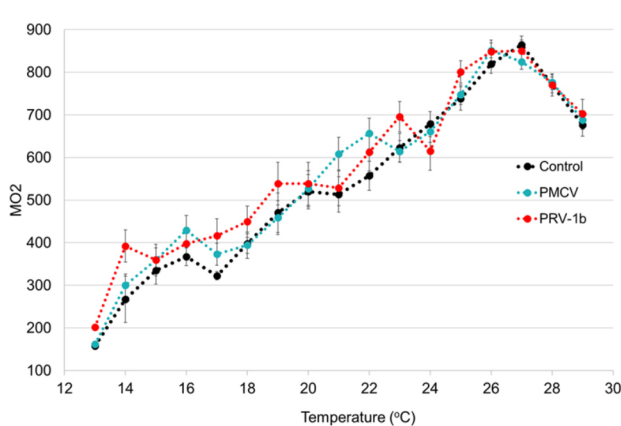
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Introduction

Viral infections associated with severe cardiac pathologies represent one of the top health challenges in Atlantic salmon aquaculture in the North Atlantic. Piscine orthoreovirus genotype 1 (PRV-1) and Piscine myocarditis virus (PMCV), induce distinct patterns of cardiac inflammation (HSMI – Heart and Skeletal Muscle Inflammation and CMS – Cardiomyopathy Syndrome) that result in compromised animal welfare and substantial production losses at sea. The association of these diseases with cardiac inflammation and impaired blood circulation raises concerns regarding physiological tolerance to different types of stress. PRV-1 variants (PRV-1a, non-virulent) endemic to the West coast of Canada cause minimal physiological impairment to infected Atlantic salmon (Zhang et al., 2019), but PRV-1 (PRV-1b, virulent) strains found in Norway reduce the capacity of infected fish to tolerate hypoxic conditions above 19°C (Lund et al., 2017). However, there is currently no literature examining the impact of PRV-1 and PMCV infection on the metabolic oxygen demand at upper thermal ranges (CTmax), particularly at the current scenario of climate change, which can make the fish more susceptible to these potential impacts. Thus, our research aimed to investigate the standard (SMR) and maximum metabolic rate (MMR), upper thermal tolerance, and relative ventricular mass (RVM) of Atlantic salmon at the peak of PRV-1b and PMCV infection.

Materials and Methods

To assess the thermal oxygen-use performance, fish infected with PMCV and PRV-1b infection were placed in a controlled respirometry chamber. The experiments took place from 8-10 weeks post-challenge (wpc), when heart inflammation was projected to reach peak severity. Each afternoon one fish was selected from either a negative control, PRV-1b or PMCV infection tank and placed in a cylindrical respirometer to have their standard metabolic rate (SMR) calculated overnight at their holding temperature (14°C) (Rodgers et al., 2016). The next morning, fish were given a critical thermal maximum (CTmax) test (Desforges et al., 2023) targeting a temperature increase of 3°C per hour, with oxygen consumption recorded every increase of 1°C, until fish lost equilibrium. The temperature reached at the loss of equilibrium was recorded as the CTmax. A total of 15 fish from each group were used across the three weeks of respirometry experiments. Fish remaining at the end of the 14wpc study were lethally sampled to have body weight and ventricle weight recorded for RVM calculations. Histology was also used to assess the timing and severity of cardiac lesions.



Group	Control	PRV-1b	PMCV
Weight (g) ± SD	215.0 ± 34.0	210.3 ± 40.1	217.9 ± 35.0
SMR ± SD	182.2 ± 27.3	194.4 ± 47.4	192.8 ± 36.0
MMR ± SD	915.1 ± 112.6	951.8 ± 107.5	923.2 ± 75.1
RVM ± SD	0.1050 ± 0.009	0.1087 ± 0.017	0.1265 ± 0.021
Welch's t-test			
P-value	-	0.3700	<0.0001

Ctmax (C°)			
Week	PRV-1b	PMCV	Control
8	28.75	28.93	29.20
9	29.00	28.83	28.89
10	29.15	29.12	28.98

Figure 1. Average Metabolic Oxygen Consumption (MO2) of Atlantic salmon from control, PMCV, or PRV-1b infection groups calculated in controlled respirometry experiments. Table 1. Average respirometry parameters.

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Results

At rest and under thermal challenge, viral infections failed to have a significant impact on the standard metabolic rate (SMR) and maximum metabolic rate (MMR) of post-smolt Atlantic salmon. However, the relative ventricular mass (RVM) of fish infected with PMCV was statistically higher than the negative control. The CT_{max} was also similar across study weeks. The trend in metabolic oxygen consumption (MO₂) with increasing temperature and respiratory parameters can be found in Figure 1 and Table 1.

Discussion

Despite the expected impact of PRV-1b and PMCV infections on cardiac function, our results indicate that neither virus significantly altered SMR, MMR, or thermal oxygen-use performance under acute warming conditions. This suggests that infected fish maintained aerobic capacity even at upper thermal limits. The preservation of metabolic performance likely reflects the activation of compensatory mechanisms across several physiological adaptations. At the blood level, splenic contraction might increase hemoglobin (HGB) and hematocrit (HCT), thereby enhancing oxygen-carrying capacity. Ventilatory adjustments, including increased rate and amplitude, could improve oxygen uptake at the gill. Cardiovascular compensation through modulation of heart rate and stroke volume may sustain cardiac output despite inflammation, while acid–base regulation (via carbonic anhydrase activity and the Bohr and Root effects) likely facilitates efficient oxygen unloading at the tissues. Interestingly, the increased relative ventricular mass observed in PMCV-infected fish may represent a structural adaptation supporting cardiac output under inflammatory stress. Together, these responses may explain why no impairment in MO₂ or CT_{max} was detected, even at peak infection. Although hypoxia adaptations are different, these findings contrast with previous reports of reduced hypoxia tolerance in virulent PRV infections, suggesting that, under the conditions tested, Atlantic salmon can buffer the functional consequences of potential cardiac damage caused by PRV and PMCV during acute thermal challenges. However, the absence of performance impairment does not preclude subclinical limitations, particularly under chronic or multifactorial stressors, such as those encountered in field conditions.

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AN AUTOMATED PROTOTYPE OF UPWELLING VERTICAL INCUBATOR FOR RAINBOW TROUT (*Oncorhynchus mykiss*) EMBRYOS TO REDUCE FOOD LOSSES DURING EARLY DEVELOPMENT

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Introduction

Rainbow trout (*Oncorhynchus mykiss*) is the main freshwater species farmed at European level, both in terms of volume and market value, where it contributed 17.7% of the overall aquaculture production in 2023, largely supported by Italy (19%), Denmark (17%), and France (14%). Monoculture is the most common rainbow trout farming, and intensive systems are considered necessary in most situations to make the operation economically attractive. In this context, the production cycle can be divided in 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 time, allowing farmers to minimize losses within the supply chain. However, the early embryonic development, from fertilization to the eyed-egg stage, still remains a critical bottleneck, since embryos are extremely sensitive to mechanical stress and handling. For that reason, developing embryos are left undisturbed in vertical flow incubators, and are only subjected to visual inspections. In this phase, the primary challenge is the high mortality caused by infections caused by water molds (mainly *Saprolegnia spp.*). 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 result infected after the visual inspection, the entire batch 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 able to gently lift the embryos and to promptly recognize (through multi-spectral cameras) and remove the non-developing or infected ones, in light to reduce possible food losses deriving from the whole batch discard.

Materials and Methods

In a preliminary stage, 50 batches of both viable (orange) and non-viable (white) rainbow trout embryos, obtained from an Italian supplier, were analyzed with XIMEA multi-spectral cameras. Particularly, embryos from each batch 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 embryos' layer with water to simulate the state of immersion. The collected images (3 images per batch with and without water) were labelled by domain experts using Labelbox platform and then employed to train a deep learning model able to detect eggs and assign label based on reflectance data. This preliminary phase was necessary to identify (spectral) bands of interest.

The prototype of the vertical incubator was developed starting from the conventional structure made of a plexiglass cylinder, implemented with a motorized screw conveyor, set at a 45° angle inside the cylinder, to lift the embryos from the bottom to the top of the incubator. At the exit of the screw conveyor, a dedicated slide mechanism was placed to accurately convey embryos from the incubation area to the classification and sorting area. The vision system, responsible for embryos' classification, was constituted by high-resolution Basler USB 3.0 RGB camera. Image acquisition was managed by a GPU-equipped computer to accelerate the inference time of the deep learning model used for detecting healthy and diseased embryos based on You Only Look Once (YOLO) model (v11). The sorting was carried out by a specialized device developed for ensure the precise removal of non-viable or infected eggs based on data acquired by the imaging system. Finally, the prototype was also integrated with a Pacific system for the continuous monitoring of water parameters (pH, temperature, dissolved oxygen, and CO₂), and a water flow meter. The prototype was tested under real farming conditions at the Società Agricola Trofocoltura F.lli Leonardi (Saone, Italy). Eggs were manually obtained from broodstock and,

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immediately after the fertilization, were divided in two different batches of 600 newly fertilized embryos each (one for the prototype and one for a traditional vertical incubator). Both batches were maintained in their respective incubator system up to the achievement of the eyed-egg stage. During this period, embryos located in the prototype, daily underwent one complete passage through the screw conveyor and camera inspection for selection. The non-viable embryos were discarded by the sorting system, collected and counted. After this testing period, eyed embryos from both batches were transferred to separate conventional hatchery systems until hatching. This experimental set up was run in triplicate.

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. The 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. Results from mortality rates during the early development indicated that the prototype was able to improve up to 5% the number of embryos that achieved the eyed stage, thus reducing losses and improving the whole productivity of rainbow trout farms. 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. Finally, 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. However, a bottleneck remains: the immediate scalability and adoption of this technology in commercial aquaculture settings.

Funding

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CIRCULAR BIOECONOMY ACROSS LAND AND SEA SIDESTREAM RESOURCES: THE ONE EARTH PROJECT

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Introduction

Aquaculture currently represents the primary source of seafood worldwide, with farmed fish playing a fundamental role in food security, providing proteins of high biological value, essential fatty acids, as well as vitamins and minerals indispensable for human health. In recent decades, the significant expansion of this sector has led to an increased demand for feed raw materials, including those of marine origin, highlighting growing critical issues related to sustainability and ecosystem impact. To address these challenges, marine-derived protein sources (like fish meal) have been progressively replaced with more sustainable alternatives, including plant proteins sources, meals derived from insects and animal by-products, and microbial biomasses (yeasts and bacteria). This process has significantly reduced reliance on wild fish stocks while maintaining adequate production performance. Conversely, replacing the marine-derived lipid fraction, specifically fish oil, has proven more complex, since it remains the primary source of long-chain n3-polyunsaturated fatty acids (n3 lc-PUFA). These molecules are essential not only for the growth, development, and reproduction of farmed fish, but also for ensuring the high nutritional value of the product intended for human consumption. To date, n3 lc-PUFA sources in aquaculture feeds still largely depend on fish oil and only marginally on alternatives such as vegetable oils (soy, rapeseed, linseed) and microalgae-derived oils that still present significant limitations. In this scenario, a particularly promising approach is represented by the use of microorganisms, such as yeasts and bacteria, which can be cultivated in controlled environments and are characterized by rapid growth rates and high production yields. These systems allow for the continuous production of n3 lc-PUFA, independently of seasonal variations and the availability of natural resources. Furthermore, from a circular economy perspective, these microorganisms can be cultivated using organic by-products from the agri-food industries, contributing to the valorization of waste and the production of biomass with high nutritional value.

Materials and Methods

The ONE EARTH project, funded by the European Union under the Horizon Europe program, fits into this context, focusing on utilizing organic residues from terrestrial agri-food supply chains as a substrate for producing high-value-added bioactive compounds. This approach aligns fully with the paradigm of the circular bioeconomy, where the by-products of one production process become resources for other supply chains, contributing to the reduction of waste and overall environmental impact. Within the project, the Università Politecnica delle Marche plays a key role, optimizing the production of volatile fatty acids (VFAs) from organic residues through anaerobic fermentation processes and evaluating the effects of a new generation of feeds on welfare and quality of commercially relevant fish species for Mediterranean aquaculture (rainbow trout and European seabass). The pilot plant consists of a whey storage system refrigerated at 4 °C, a 500-liter fermenter, and a membrane separation unit for ultrafiltration. Inside the fermenter, the whey undergoes controlled microbial fermentation, during which selected microbial communities convert Dairy industry by-products into VFAs and other metabolic intermediates. Ultrafiltration then allows for the separation and concentration of the fractions of interest, improving the overall efficiency and reproducibility of the process at a pilot scale. The obtained VFAs represent a strategic carbon platform for fueling the growth of oleaginous microorganisms, including marine bacteria and yeasts such as *Yarrowia lipolytica*, capable of accumulating high amounts of n3 lc-PUFA. The most promising strains are transferred to the pilot scale to optimize cultivation conditions and maximize production yield. The obtained microbial biomass and extracted fatty acids will be subsequently used to formulate experimental fish feeds to be tested during 3-month feeding trials. The effects of experimental diets will be assessed through fish growth performance, feed conversion efficiency, gut and liver health status, and nutritional value of the edible portion.

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The ONE EARTH project also addresses the management of waste generated by the fish industry in an integrated manner. Residues such as fish heads, scales, skin, and bones represent a biomass rich in organic and mineral compounds that, if not properly valorized, constitute an environmental and economic disposal cost. In this regard, fish residues will be subjected to pyrolysis to obtain biochar (rich in Ca and P) intended as soil conditioner for agriculture. Research activities have identified the operational conditions capable of maximizing process yield while preserving the nutritional properties of the biochar. In this way, the organic matter valorization cycle within the supply chain is completed, demonstrating how by-products from different sectors can be transformed into new resources through the integration of biotechnological and thermochemical processes.

Results and Discussion

The growing global demand for seafood requires the development of solutions capable of increasing production without compromising the balance of natural ecosystems. In this context, ONE EARTH proposes an innovative model based on the integration of anaerobic fermentation, microbial biotechnologies, and thermochemical valorization processes, where the residues of one supply chain become resources for another. The expected results will provide quantitative insights into the actual feasibility of replacing fish oil with microbial biomass or microbial fatty acids in the diets of carnivorous species of Mediterranean interest. These are key elements to guide the choices of the feed industry and support the transition toward more sustainable aquaculture systems that are less dependent on marine resources.

Funding

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A ONE-SHOT COMPUTER VISION FRAMEWORK FOR INDIVIDUAL FISH IDENTIFICATION IN PRECISION AQUACULTURE

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Introduction

Reliable identification of individual fish is a fundamental requirement for aquaculture research and production, particularly when individual-level monitoring is needed for biomass estimation, health assessment, selective breeding, and growth tracking. However, in many aquaculture settings, individual fish are still monitored through group-based observations because conventional identification approaches often rely on physical tagging. These methods are labour-intensive, time-consuming, impractical for large-scale systems, and in some cases infeasible because handling or tagging may interfere with experimental conditions, disrupt normal production, or negatively affect fish welfare.

Although several non-invasive image-based identification approaches have been proposed, many of them have mainly been evaluated under controlled or short-term experimental conditions and are not yet sufficiently validated under realistic longitudinal aquaculture scenarios [1]. Moreover, many existing approaches depend on having a relatively large number of images per individual, which is often unrealistic in practical aquaculture experiments. In real monitoring conditions, only a limited number of images may be available for each fish, while appearance can also change over time due to growth, health status, pose variation, and differences in imaging conditions. This limited-image-per-individual setting therefore remains a major challenge for reliable fish identification.

Recent advances in computer vision and deep learning provide a promising basis for overcoming these limitations by enabling image-based identification of individual fish without physical contact. In this context, methods that can maintain high identification accuracy when only a small number of images per fish are available are particularly important. Image-based individual identification therefore offers a non-invasive alternative to conventional tagging methods, enabling remote, rapid, and cost-effective in situ monitoring, while also requiring careful consideration of ethical issues such as animal welfare.

Materials and Methods

In this study, a prototype-based one-shot individual identification framework was developed for early juvenile *Oncorhynchus mykiss*. A ConvNeXt backbone network, pre-trained on a longitudinal image dataset of rainbow trout, was used as the main feature extractor and enhanced with a multi-stage feature fusion strategy to obtain discriminative visual representations from fish images.

In this framework, the pre-trained model encoded each fish image into a latent embedding space, where individual-specific visual patterns were represented as compact identity descriptors. Prototype representations were generated for each individual using a single reference image, consistent with the one-shot identification setting. To improve prototype robustness, the reference image was further expanded using synthetic augmentation. During inference, query images collected after a temporal gap at subsequent sampling sessions were compared with the stored prototypes using cosine similarity. Identity was assigned according to the prototype with the highest cosine similarity score.

The proposed method was evaluated using a long-term red mark syndrome (RMS) monitoring dataset consisting of 138 individual rainbow trout. For each individual fish, single images of the left and right lateral sides were photographed under controlled imaging conditions in a photo tent. This dataset was collected over 99 days, which includes five sampling events of photos throughout the duration of the experiment. During inference, new observations from later sessions were matched against prototypes generated from previously observed samples. Identification was evaluated by comparing the predicted identity of each query image with its known ground-truth identity. In addition, predictions from the left and right lateral sides were assessed jointly. A final individual-level prediction was considered correct only when both lateral views were assigned to the same identity and this identity matched the ground-truth label. This evaluation design enabled assessment of the framework's ability to maintain individual recognition under longitudinal monitoring conditions with temporal variation in fish appearance.

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Results and Discussion

The proposed one-shot identification framework was evaluated using Rank-1 accuracy across consecutive photo-sampling sessions. For each query image collected in a later session, identity prediction was performed by comparing its embedding with prototype representations generated from the previous session. Left and right lateral images of each fish were first evaluated separately and then jointly assessed to confirm individual identity. A prediction was considered correct only when the identities retrieved from both lateral views were consistent and matched the known ground-truth identity. Ground-truth labels were verified using recorded individual information and expert visual assessment. Between all sequential sampling sessions, this method achieved an average 97.71% Rank-1 accuracy. This confirms reliable identification performance despite changes in fish appearance, including growth-related morphological variation and lesion-associated changes caused by red mark syndrome [2]. The outcome results indicate that the proposed one-shot framework can reliably identify individual fish despite changes in appearance. These findings indicate its potential use in aquaculture research and production, particularly for health monitoring, growth tracking, and welfare-oriented management.

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FARMED BLUE MUSSELS: LOW-IMPACT FOOD DESPITE INCLUSION OF EMISSIONS FROM SHELL FORMATION

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Introduction

Aquatic foods are increasingly recognised as key components of sustainable and nutritious diets, with unfed bivalves such as mussels among the lowest-impact animal protein sources (Parodi et al. 2018, Gephart et al. 2021). The Mussel Puzzle is a three-year (2024-2026) research project, quantifying life cycle environmental impacts of farmed blue mussels across North Atlantic sites. Here, we present a holistic appraisal of the environmental performance of northeast Atlantic blue mussel aquaculture, including its variability across production areas and technologies, and evaluate impact drivers and improvement options. Insights on carbonate chemistry and shell formation are integrated by adding estimated production-area-specific CO₂ released from mussel shell formation as potential additional life cycle emissions.

Methods

LCA data addressing production technique, use and lifetime of materials, boat use and fate of the biomass harvested, was collected from four farming systems for 2021-2024 through online and face-to-face meetings with mussel producers. Greenhouse gas emissions, marine eutrophication and land use were quantified per kg of live-weight mussels. Biogenic CO₂ emissions from shell formation were calculated using shell CaCO₃ content, a molecular conversion factor, and seawater buffering capacity derived from location-specific oceanographic data.

Results and discussion

Variability in environmental impact drivers was found between the four farming systems, reflecting differences in environmental conditions as well as farming strategies and technical solutions. Greenhouse gas emissions of the two systems using a reseeding technique were lower than the ones applying natural settling. Fuel use in growout was an important driver of greenhouse gas emissions across all systems, which makes reduction efforts and shifting to alternative fuels important. Reseeding systems, on the other hand, had the highest eutrophication and land use impacts and minimising the use of virgin cotton sock (e.g. by using recycled cotton) or shifting to alternative materials could represent an improvement option, unless introducing trade-offs. CO₂ biocalcification emissions were relatively even across all sites and could, when added to life cycle greenhouse gas emissions, significantly increase total emissions, depending on how much CO₂ actually reaches the atmosphere. Even if we assume that 100% of these biogenic emissions reach the atmosphere, blue mussels are still a low-emission type of food compared to other protein sources, whether seafood or from land-based animal production systems (Bianchi et al. 2022).

Conclusions

There are differences in environmental impacts between mussel farming techniques and locations, which opens up opportunities for improvements of this already low-impact production system. Shell formation could release significant amounts of CO₂ to the atmosphere relative to life cycle greenhouse gas emissions, but to date, the exact extent is unknown. However, despite adding these additional emissions as a worst-case scenario, mussels remain a low-impact animal-source food.

Acknowledgment

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A ROADMAP TO UPSCALE LOW-TROPHIC AQUACULTURE IN OFFSHORE WINDFARMS INSIGHTS FROM THE ULTFARMS PROJECT

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Co-locating low-trophic aquaculture (LTA) with offshore wind farms (OWFs) can unlock new economic opportunities and make better use of marine space, yet uptake is often constrained by fragmented permitting, demanding offshore operations and the need for credible environmental evidence. [ULTFARMS](#) (2023–2026) is a 42-month Horizon Europe project led by [Deltares](#), bringing together 25 partners from nine countries and six pilots in the North Sea and Baltic Sea. The project integrates engineering, technical, and ecological innovations for seaweed, mussel and oyster systems, while also exploring links with nature-inclusive design and restoration activities.

Building on project outputs and insights, ULTFARMS develops an actionable roadmap to translate this experience into clear guidance for policymakers, industry and research, lowering barriers and supporting short-term replicability of offshore LTA in OWFs. The roadmap is organised as a main roadmap comprising standalone thematic shorter roadmaps, each designed to be read independently and to provide practical steps and keys to success. It addresses licensing, operations and maintenance, finance and funding, monitoring and indicators, upscaling and commercialisation, and advancing eco-friendly productivity, and it concludes with lessons learned and a way forward.

The ULTFARMS roadmap also draws on key outputs from the sister project [OLAMUR](#) to strengthen the evidence base and practical guidance for upscaling offshore LTA in OWFs.

RETHINKING THE RESPONSIBLE APPLICATION OF NATURE-INCLUSIVE DESIGN IN MARINE INFRASTRUCTURE TO RESTORE ECOSYSTEMS AND ENHANCE BIODIVERSITY

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Nature-inclusive design (NID) of marine infrastructure refers to the creation of optimized artificial habitat to locally enhance biodiversity. In essence, NID comprises the local addition of something non-natural to infrastructure with the aim of providing habitat for biodiversity assets in its widest sense. In its purest sense, NID is inspired by how the local environment would be without the infrastructure being present. However, NIDs of marine infrastructure often comprise actions that promote biodiversity which does or did not naturally occur at that location.

In this presentation, we review existing definitions of NID and introduce the perspective of the ULTFARMS project consortium (Degraer et al., 2026). We propose a clear distinction between three different types of NID: (1) restorative NID, linked to the restoration of lost local ecosystem values, and (2) creative NID, referring to artificially created ecosystem assets and containing either (2a) optimized infrastructure or (2b) add-on structures. Restorative NID refers to measures which facilitate and speed-up nature restoration, while creative NID refers to measures to boost biodiversity “beyond the natural”.

Beyond the importance of distinguishing different types of NID, it is important to also reflect on which biodiversity assets we elect to boost. We argue that NID should move beyond a focus on species richness towards supporting functional ecosystem assets, which also provide better options for climate-proof NID designs. To adopt NID in an offshore renewables context, it is imperative to hold our horses and carefully consider what is the target to be achieved and how to get there. Moreover, we highlight that NID interventions carry potential negative impacts such as natural habitat loss, the spread of non-indigenous species and unintended shifts in the food web, with the latter issue being strongly scale-dependent. These risks need to be weighed against the expected ecological benefits. Applying NID in offshore renewable energy therefore requires clear goals, careful selection of the ecological assets that we aim to promote and consideration of potential trade-offs.

In summary, restorative NID can be actively promoted, whereas creative NID requires more careful reflection; NID preferably prioritizes functional ecosystem assets and explicitly considers which ecological values to promote and why.

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MODELLING LOW-TROPHIC AQUACULTURE CO-LOCATED WITHIN OFFSHORE WIND FARMS IN THE NORTH SEA UNDER CLIMATE CHANGE SCENARIOS

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There are currently plans to upscale the cultivation of shellfish and seaweed to offshore areas in the North Sea, as part of the envisioned Blue Economy and as a promising option for multi-use of offshore wind farms. Previous research has shown that monopiles of offshore wind farms locally affect hydrodynamics and water quality, influencing local nutrient availability, the light climate and hence primary production. However, there is still limited knowledge on the implications of these changes for aquaculture species growing within offshore wind farms, and how this interaction will change under future climate conditions.

As part of the EU-funded ULTFARMS project, we modelled large-scale cultivation of sugar kelp and/or blue mussels, co-located within offshore wind farms in the North Sea. A state-of-the-art ecosystem model was used to determine the local interplay between offshore wind farms and aquaculture sites under different climate scenarios, including the interaction of the wind turbines with ambient flow. To also include the effects of offshore wind farms on the wave field and hence on fine sediment dynamics, the spectral wave model DCSM-SWAN was employed. The wave model was run for two scenarios with and without wind farms both for historical and future periods.

The SSP5-RCP8.5 climate change scenario, representing highest greenhouse gas emissions, was selected. Policy-driven changes in river input, affecting nutrient loads, were also incorporated. Simulations were run under the projected climate conditions around 2050, in line with the 2050 horizon of the European Green Deal. To assess pure climate change effects, the results were compared with a historical reference period around 2005.

This presentation will provide insights 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 their implications for the growth of sugar kelp and blue mussels. These 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.

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IMPROVING THE ENVIRONMENTAL PERFORMANCE OF RAINBOW TROUT (*Oncorhynchus mykiss*) FARMING THROUGH INSECT-BASED FEEDS: INSIGHTS FROM NEWRIFF AND HIGA PROJECTS

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Introduction

Aquaculture is one of the fastest growing food production sectors, with feed production representing a major contributor to its environmental impact. The reliance on fishmeal and other conventional protein sources raises concerns regarding resource use and long-term sustainability. In this context, insect-derived ingredients have emerged as promising alternatives, as they can valorise organic side-streams while reducing dependence on traditional feed resources (Gasco et al., 2023).

This study combines results from the newRIFF and HIGA projects to evaluate the environmental performance of using *Hermetia illucens* (HI) meal in diets for rainbow trout (*Oncorhynchus mykiss*) through a Life Cycle Assessment (LCA) approach.

Materials and methods

In the newRIFF project, a benchmark LCA was performed on a conventional Italian trout farm using a cradle-to-farm gate approach and 1 t of live fish as functional unit. Primary data were collected from an industrial-scale trout farm and included feed use, energy consumption, oxygen use and infrastructure. Emissions to water were estimated as secondary data through mass balance modelling. Additionally, alternative feed scenarios were formulated by partially replacing fishmeal (5% and 10%) with HI meal produced from locally available agri-food by-products.

Within the HIGA project, a feeding trial was conducted using six experimental diets containing 10% HI meal. Four diets included increasing levels of chitinase (0–0.75%), while two diets used thermally pre-treated HI meal, with or without enzyme addition. A total of 480 juvenile trout (initial average weight of 16 g) were reared in 24 tanks over 74 days, reaching three times their initial body weight at the end of the trial. Growth performance, feed intake and feed conversion ratio (FCR) were monitored and used as primary data to build the Life Cycle Inventory (LCI). The LCA was applied using a cradle-to-farm gate approach with 1 kg of trout weight gain as functional unit.

Environmental performance of feeds was estimated based on ingredient composition and literature datasets (e.g., Ecoinvent), while HI meal production was modelled using a combination of primary data (substrate composition and rearing efficiency) and secondary data for energy use and emissions.

Results and discussion

The LCA of the conventional trout farm showed a climate change impact of approximately 1,980 kg CO₂ eq t⁻¹ of live fish, in line with literature values for flow-through systems. Feed production was identified as the main hotspot, contributing around 50% of the climate change impact and up to 90% in some impact categories such as land use.

The comparison of feeds in the newRIFF framework showed differences generally within ±10% across all impact categories. Diets including insect meal (5–10%) showed an increase in climate change impact ranging from +3% to +8% and in fossil resource use up to +10%, while reductions were observed for Net Primary Production Use (−10% for 5% inclusion and −20% for 10% inclusion).

When integrating the HIGA experimental parameters into the LCA model, the similar growth performance observed across diets resulted in comparable environmental impacts per kg of fish produced. In particular, differences in FCR within the observed range (0.91–0.98) translated into variations in climate change impact of approximately −5% to +6% compared to the baseline diet scenario (standard insect meal without chitinase). Lower FCR values were associated with reduced feed demand and consequently lower impacts.

Overall, the partial replacement of fishmeal with HI meal, under equivalent productive performance conditions, resulted in stable or moderately improved environmental performances. Benefits were mainly associated with resource-related indicators and circularity aspects, especially when low-impact side-streams were used as insect substrates. However, energy use during insect production remains a key factor influencing climate change impact.

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Conclusions

This study provides integrated insights into the use of insect meal in aquafeeds by combining experimental feeding trials and LCA-based modelling. Results confirm that insect meal can be included in trout diets without compromising growth performance, while offering environmental benefits under specific conditions.

However, current impacts are strongly influenced by the scale and efficiency of insect production systems. Further optimisation is needed to fully exploit the potential of this circular approach. Within the newRIFP project, feeding trials will be carried out in the coming months and will be tested through growth trials to validate these findings at full system level and enable a complete LCA of the fish production phase.

Acknowledgment

This work was supported by the newRIFP project (Fondazione Cariplo) and the HIGA project. The newRIFP project is supported within the call Circular Economy – Promoting research for a sustainable future – 2022 - funded by Fondazione Cariplo. The HiGa project is supported within the Green Era Hub call by the Green Development and Demonstration Programme, GUDP, under the Italian Ministry of Agriculture, Food Sovereignty and Forests (MASAF) (CUP: D23C23000570005).

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AN INTEGRATIVE APPROACH TO SUSTAINABLE HEALTH MANAGEMENT IN ADRIATIC MARINE AQUACULTURE

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Introduction

In Adriatic marine aquaculture, diseases remain a major challenge, negatively affecting fish health, welfare, and production efficiency. This challenge is further exacerbated by climate change, which may alter pathogen dynamics and increase the risk of disease outbreaks. In general, the period from the detection of the first clinical signs to diagnosis and initiation of appropriate treatment may last at least ten days. During this period, the disease may spread extensively within the farming unit, potentially reducing the effectiveness of treatment and increasing production losses. To promote more proactive and effective health management, the Interreg MARINET project aims to improve the sustainability and climate resilience of Adriatic marine aquaculture while addressing emerging production needs. The project combines technological and preventive approaches, including biosensors for continuous environmental monitoring, artificial intelligence-based risk assessment, enhanced biosecurity measures, and the development of innovative vaccines.

Material and Methods

Use of technology and AI in health management. Considering the environmental conditions that may favour pathogen proliferation, sensors were installed on three different farms to enable continuous monitoring of key seawater parameters. The sensor data are transmitted to and stored in a central data lake. In addition, a Progressive Web Application (PWA) was developed to allow farmers to record selected welfare indicators, feeding activities, and other daily farm operations. These data are also integrated into the data lake. Together with historical diagnostic data collected by veterinary diagnostic laboratories and images of diseased fish, these integrated datasets are used to develop an AI-driven risk assessment and early-warning model capable of identifying increased disease risk and supporting timely management decisions.

Biosecurity framework. A harmonised biosecurity document was developed based on the Italian guidelines *Buone prassi in materia di biosicurezza negli stabilimenti di acquacoltura*, approved by the Italian Chief Veterinary Officer (CVO), and on the assessment of biosecurity practices at industrial and small-scale, family-owned and operated farms. The resulting framework was designed to support the development and implementation of comprehensive, farm-specific biosecurity plans for European seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) production in the Adriatic region.

Vaccination. Vaccination was addressed as an integral component of the biosecurity strategy. Autogenous vaccines against *Vibrio harveyi* and *Tenacibaculum maritimum* were developed and evaluated under laboratory conditions. In addition, an experimental subunit vaccine formulation containing selected bacterial antigens and nano-particulate immunostimulants as adjuvants was developed as an alternative approach to support sustainable disease management in Adriatic marine aquaculture.

Results

The PWA developed for real-time monitoring and data management enables the systematic acquisition, integration, and cataloguing of data from multiple sources. These data are incorporated into AI-powered predictive models, providing a basis for improved risk assessment, decision-making, and aquaculture production management. In the current evaluation, the model achieved an accuracy of 95% for disease prediction and approximately 75% for the identification of disease-associated symptoms based solely on fish images.

A new biosecurity template, comprising a set of checklists tailored to Adriatic aquaculture farms, was developed to support farm managers in implementing and maintaining high biosecurity standards.

The newly developed vaccine formulation showed potential to enhance immune responses in fish under laboratory conditions. However, further evaluation under commercial farming conditions is required to confirm its efficacy and practical applicability.

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Conclusions

The MARINET approach integrates real-time environmental monitoring, farmer-generated data, AI-based risk assessment, and enhanced biosecurity and vaccination strategies to support more proactive health management in Adriatic marine aquaculture. Preliminary results demonstrate the potential of AI-based tools for early disease risk assessment and image-based disease and symptom identification. Further validation under commercial farming conditions is needed to confirm the performance and practical applicability of the developed tools and vaccine formulations.

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VARIATIONS IN CONDITION INDEX AND GROWTH OF *Mytilus galloprovincialis* IN THE ADRIATIC SEA

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Introduction

Mediterranean mussel *Mytilus galloprovincialis* is one of the most important aquaculture species in the Mediterranean and Adriatic Seas. Over the last decade, its production has faced several challenges, including high mortalities linked to marine heat waves and extreme summer temperatures (Lupo et al., 2021). Despite its commercial importance, relatively little is known about the spatial and temporal variations in growth rates and condition index of *M. galloprovincialis* in the Adriatic Sea, or the factors that influence them. A recent study by Peharda et al. (2024) documented cessation/slowdown of *M. galloprovincialis* growth during periods of high temperatures and reduced salinity in the Krka River estuary. However, that study was spatially limited and did not include an analysis of the condition index. Thereby, within the framework of the research project BivalveSPEECH, which uses sclerochronological methods to obtain insights into bivalve growth patterns and the environmental and biological factors influencing them, we conducted research at three aquaculture sites in the eastern Adriatic Sea.

Material and methods

Monthly samplings were conducted between March 2025 and March 2026 at three sites in the coastal area of the eastern Adriatic Sea, including Rovanjaska (Velebit Channel area), Novigrad Sea and Bistrina (Mali Ston Bay). At each site, temperature and conductivity were measured at hourly (Rovanjaska and Novigrad Sea) or 10-minute (Bistrina) resolution. Each month, 30 specimens of *Mytilus galloprovincialis* were collected from each sampling location at a depth of 4 m and transported to the lab. Condition index was expressed as the ratio of dry tissue weight (after 48 h at 60°C) to total dry shell and tissue weight.

For the growth analysis, the experiment was conducted in the Novigrad Sea. Shells (N=60) were measured in the field using a digital calliper and individually marked using Hall print shellfish tags in October 2025. They were randomly divided into two groups: the first was placed in a basket at ~1.5 m depth, while the second was placed at ~4.5 m depth. Specimens were left in place until early March 2026, when they were collected, transported to the lab, and their growth was analysed.

Results and discussion

Hydrographic conditions varied by sampling site and season. The lowest salinity conditions were observed at the sampling site in the Novigrad Sea, lasting for several months, from late November 2025 to the end of the experiment in March 2026. Temperature in general followed annual cycle, with the lowest values observed in the Novigrad Sea during winter months, particularly above the halocline, near the surface. Maximum summer temperatures surpassed 26 °C in July 2025 at all three sites. Condition index values had a clear seasonal pattern only for specimens from Bistrina (Mali Ston Bay), with the highest values observed during the summer months. Overall, analysed specimens from Rovanjaska and the Novigrad Sea had higher condition index values than those from Bistrina, and these values were relatively constant throughout most of the year. This indicates variations in reproduction between sites, with Bistrina characterised by a single major spawning peak, while at Rovanjaska and the Novigrad Sea several spawning events occurred. The majority of tagged shells in the Novigrad Sea survived the experimental period, even those exposed to low salinity. Shells placed deeper grew faster than those placed near the surface, which were exposed to less saline water. The obtained results are compared to the previous studies, including Peharda et al. (2007) on the growth of mussels in experimental integrated aquaculture. Our results contribute to understanding the ecology of *M. galloprovincialis* in aquaculture settings and provide important parameters for high-resolution shell geochemical studies. Additionally, the results indicate that farm management should be adapted in terms of cultivation depth due to the severe impact of low salinity periods on the growth and condition index of the mussels. Further research includes stable isotope analysis ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) in shell carbonate material to obtain insight into the seasonality of growth and the effects of reduced salinity on the growth dynamics of *M. galloprovincialis*.

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THE MEDITERRANEAN SCALLOP *Pecten jacobaeus* L. GROWTH IN LANTERN NETS IN THE KRKA RIVER ESTUARY (CROATIA)

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Introduction

Mariculture is the primary source of bivalves globally, but this supply is often limited to a small number of species. In the Mediterranean Sea, the primary bivalves farmed are mussels and oysters, highlighting the need to introduce new species. The Mediterranean scallop (*Pecten jacobaeus* Linnaeus 1758) is a highly valued commercial species with strong market demand that currently exceeds available supply from fishing. According to previous research, *Pecten jacobaeus* can reach market size in just 18 months, with high survival rates, making it a potential candidate for aquaculture.

The aim of this study was to monitor *P. jacobaeus* growth in suspended installations - lantern nets - for future aquaculture purposes.

Materials and methods

Scallop spat collected with mesh bag collectors from the Krka River estuary in October 2024 were measured for shell length and then placed in lantern nets. In February 2025, juveniles were tagged with numbered markers and separated in lantern nets at two depths, at 8 and 15 m. Each month, juveniles were measured to estimate their growth, specifically shell length, height, and width, each recorded to a precision of up to 1 mm. During the experiment, environmental parameters, temperature and salinity were monitored.

Results

Our results indicate that growth of *P. jacobaeus* juveniles from the Krka River estuary is rapid, and commercial size can be achieved after approximately 18 months. Growth rate was fastest during their earliest life stages at the beginning of the research, when juveniles were at the smallest sizes and decreased as they approached adult sizes. The fastest growth rate was recorded during the spring period, while a slowdown in growth was recorded during winter and summer. Juveniles placed in lantern nets at lower depth exhibited better growth overall compared to those at greater depth, although growth also varied seasonally by depth. The survival rate was low overall, with the highest mortalities recorded at the start of the research when the spat was at the smallest sizes but decreased as they grew larger.

Discussion

The fastest growth rate of juvenile *P. jacobaeus* during spat stages is due to energy being dedicated completely to rapid growth, while it slows down as energy also shifts to reproduction as they reach adult size. Fast growth rates during spring can be linked to optimal temperature and food conditions, which peak during this season, while the slowdown of growth during winter is mainly due to unfavourable conditions, mainly too low or too high temperatures. Greatest mortality rates were observed among the smallest juveniles, as they are most vulnerable during handling because of their thin shell and fragility, but the mortality rate decreased as juveniles grew larger and became more resilient. High mortality rates also coincided with unfavorable environmental conditions, such as salinity decline in the spring and too high temperatures during summer.

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ADDENDUM

EFFECTS OF DIETARY ADDITIVES ON PHYSIOLOGICAL 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 B₁₂ 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 CoCl₂ (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 CoCl₂ 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 CoCl₂ 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 CoCl₂ + 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 B₁₂ (Tonye & Sikoki, 2014) and the role of vit C in iron absorption and erythropoiesis (Garcia *et al.*, 2007). Vitamin B₁₂ 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 CoCl₂ + 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 CoCl₂ in the hematology and health of Siberian sturgeon.

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Treatments	Parameters			
	WBC (mm ⁻³)	RBC (× 10 ³ mm ⁻³)	Hemoglobin (g dl ⁻¹)	Hematocrit (%)
Control	9533.3 ± 102.7 ^f	641.3 ± 6.1 ^d	4.9 ± 0.0 ^c	30.3 ± 0.6 ^{bcd}
2 CoCl ₂	11466.7 ± 102.7 ^{dc}	637.7 ± 11.4 ^d	4.6 ± 0.1 ^d	29.7 ± 0.8 ^{cd}
4 CoCl ₂	12466.7 ± 531.2 ^{bcd}	670.3 ± 5.9 ^{bc}	5.3 ± 0.1 ^b	31.3 ± 0.6 ^{bc}
200 AA	12800.0 ± 933.6 ^{bc}	659.3 ± 11.6 ^{bcd}	5.2 ± 0.0 ^b	30.3 ± 0.2 ^{bcd}
800 AA	1525.0 ± 54.0 ^a	679.7 ± 2.7 ^b	5.3 ± 0.0 ^b	31.7 ± 0.2 ^b
2 CoCl ₂ + 200 AA	11640.0 ± 88.3 ^{cde}	711.7 ± 7.2 ^a	5.4 ± 0.0 ^a	33.7 ± 0.6 ^a
4 CoCl ₂ + 800 AA	13266.7 ± 490.5 ^b	678.3 ± 6.7 ^b	5.2 ± 0.0 ^b	30.0 ± 0.4 ^{bcd}
2 CoCl ₂ + 800 AA	13033.3 ± 352.0 ^b	656.0 ± 5.2 ^{bcd}	5.2 ± 0.0 ^b	29.0 ± 0.4 ^d
4 CoCl ₂ + 200 AA	10666.7 ± 143.4 ^{ef}	648.7 ± 9.0 ^{cd}	5.2 ± 0.1 ^b	31.0 ± 0.8 ^{bc}

Treatments	Parameters			
	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)
Control	11.0 ± 0.8 ^b	88.3 ± 1.6 ^a	2.0 ± 0.4 ^b	0.0 ± 0.0 ^b
2 CoCl ₂	11.0 ± 1.1 ^b	86.7 ± 2.6 ^a	5.0 ± 0.4 ^a	0.0 ± 0.0 ^b
4 CoCl ₂	13.3 ± 0.9 ^{ab}	84.3 ± 2.7 ^a	5.0 ± 0.4 ^a	0.4 ± 0.2 ^{ab}
200 AA	13 ± 1.1 ^{ab}	82.3 ± 2.0 ^{ab}	5.3 ± 0.5 ^a	0.0 ± 0.0 ^b
800 AA	14.3 ± 0.6 ^a	77.7 ± 1.2 ^b	5.0 ± 0.4 ^a	0.8 ± 0.3 ^a
2 CoCl ₂ + 200 AA	12.0 ± 0.4 ^{ab}	82.3 ± 0.8 ^{ab}	4.0 ± 0.0 ^a	0.0 ± 0.0 ^b
4 CoCl ₂ + 800 AA	11.3 ± 0.6 ^b	83.3 ± 0.6 ^{ab}	4.7 ± 0.6 ^a	0.0 ± 0.0 ^b
2 CoCl ₂ + 800 AA	11.7 ± 0.6 ^b	77.7 ± 2.1 ^b	5.3 ± 0.6 ^a	1.0 ± 0.6 ^a
4 CoCl ₂ + 200 AA	11.0 ± 1.7 ^b	86.3 ± 1.6 ^a	4.0 ± 0.8 ^a	0.0 ± 0.0 ^b

Two- Way ANOVA								
	WBC	RBC	Hemoglobin	Hematocrit	Neutrophil	Lymphocyte	Monocyte	Eosinophil
CoCl ₂	0.338	0.423	0.003*	1.000	0.147	0.255	0.280	0.527
Ascorbic acid	0.000*	0.000*	0.000*	0.004*	0.558	0.000*	0.072	0.009*
Ascorbic acid + CoCl ₂	0.000*	0.003*	0.000*	0.000*	0.015*	0.095	0.001*	0.044*

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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FROM AQUACULTURE NUTRIENTS TO VALUABLE BIOMASS:
CIRCULAR AQUACULTURE AT EUROPE’S LARGEST DUCKWEED CULTIVATION SITE

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Introduction

Aquaculture requires practical approaches that recover nutrients, improve water quality and create value from biological resources. Mount Lucas, Co. Offaly, Ireland, hosts Europe’s largest duckweed cultivation site and is being developed through CIRCULAR-IMTA-DEMO as a full-scale circular aquaculture living laboratory. The project integrates Integrated Multi-Trophic Aquaculture (IMTA), bioremediation and biomass valorisation in a closed-loop system where outputs from one biological stage become inputs for another.

Closed-loop Integrated Multi-Trophic Aquaculture

Rainbow trout ponds generate nutrient-rich water containing nitrogen and phosphorus. Water flows towards a 16-channel treatment lagoon where duckweed uses these dissolved nutrients to produce biomass while contributing to natural water treatment. Treated water returns towards the aquaculture ponds and the cycle begins again. Harvested duckweed can be valorised through feed, protein ingredient, nutraceutical and biorefinery pathways. The system therefore converts aquaculture nutrients into a productive second biomass stream while closing the water and nutrient loop.

Demonstration and significance

Mount Lucas enables aquaculture production, nutrient recovery, biomass generation and valorisation to be assessed as one connected system. Water-quality and environmental sensing, drones, Earth observation, analytics, open data and a digital-twin environment support measurement and validation under operational conditions. The circular logic is simple: fish generate nutrients; nutrients grow duckweed; duckweed supports water treatment; biomass enters new value chains; and treated water returns through the system. At Europe’s largest duckweed cultivation site, CIRCULAR-IMTA-DEMO provides a platform for investigating a measurable, fully circular and potentially replicable IMTA model.

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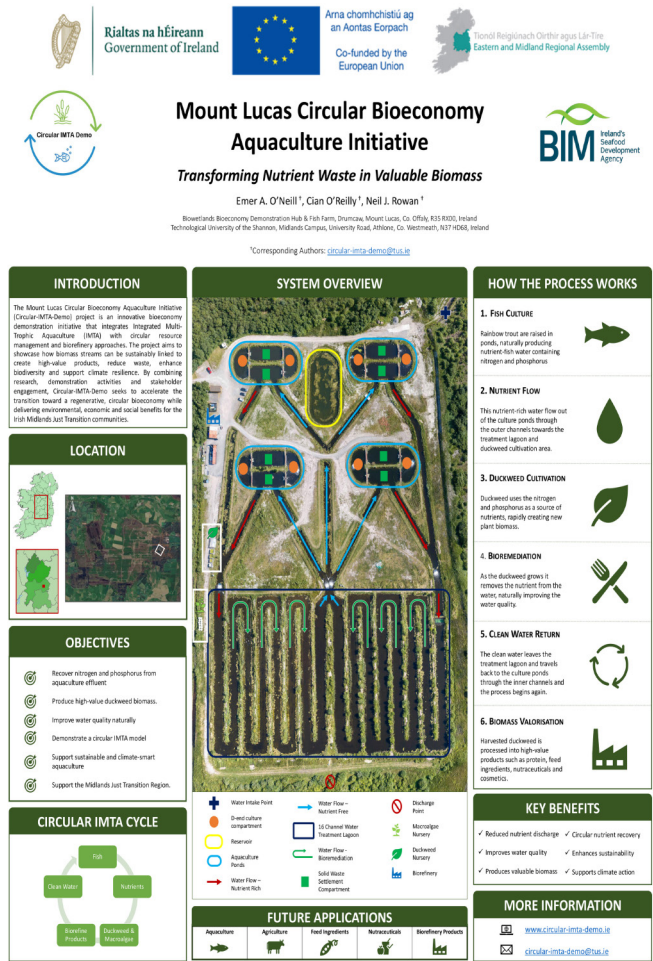


Figure 1. Mount Lucas circular bioeconomy aquaculture demonstration system and closed-loop nutrient pathway.